POLLENIA CALAMISESSA, n. sp. Text-fig. 2.

On the male the eyes are approximate for a considerable distance; those of the female are wide apart, the frons being normal in width. The head is black with a grey pulverulent covering seen from above. Most of the hairs on the male are black, on the female yellow, and in other respects the characters conform to those of *P. flindersi*, with the antennae of the male mainly black.

The thorax dorsally is shining black, with black hairs and bristles. The latter correspond to those of *P. flindersi*, except there are three postsutural acrostichals and only three marginal bristles on the scutellum of both sexes. There is a strong trace of grey pulverulent covering over the dorsum, and this is weakly continued over the black shining abdomen. The pleura has traces of yellow hair in places, some of these being crinkly, but on the abdomen and the legs all hairs are black.

The aedeagus is short and stouter than that of *P. flindersi*, the apical part beyond the slender strut being conspicuously so, but the claspers and forceps compare with those on that species and the accessory plates are much narrower, being only half the width.

Hab.—Queensland: Brisbane, 10 males, 2 females, December, 1929. All these were taken resting on reeds along a watercourse near a permanent waterhole, 15 miles from Brisbane, along the Southport Road. In this same spot, and with the same habit, another species of *Pollenia* was taken in October, 1927.

THE EMBRYOLOGY AND SEEDLING DEVELOPMENT OF *AEGICERAS MAJUS* GAERTN.

By Gladys Carey, B.Sc., Demonstrator in Botany, University of Sydney, and Lilian Fraser,* M.Sc., Linnean Macleay Fellow of the Society in Botany.

(Thirty-two Text-figures.)

[Read 26th October, 1932.]

Introduction.

For many years vivipary has been recognized as a feature of the mangroves of the world. Treub (1883) was the first to undertake an investigation of the development of the seedlings by tracing the embryology of *Avicennia officinalis* Linn. in all its stages. Later Haberlandt (1896) examined the methods of nutrition of the viviparous embryos and the seedlings of *Bruguiera eriopetala*, *Rhizophora mucronata* and *Aegiceras majus*. His observations regarding *Aegiceras majus* were made on material in which the youngest ovule was already 7 mm. long. The writers, having had access to much younger material, find that the early stages in the development of the seed show several additional points of interest. These, combined with Haberlandt's detailed account of the older embryo, give a complete account of the growth of the seed and seedling of this species. In addition, they afford an interesting comparison with the stages of development in *Avicennia officinalis* as described by Treub.

Aegiceras majus Gaertn. is a low shrub or small tree. It is frequently found associated with *Avicennia officinalis* Linn. on the marshy flats or the sloping banks of the coastal rivers in the Sydney district. This has already been recorded by Collins (1921). Where it is present in any abundance it may form a definite belt behind a zone of *Avicennia*. The size of the plant varies considerably, and there appear to be two fairly well defined growth forms present. Those plants which grow on the tidal flats skirting the coastal harbours, and whose roots are hence swept twice daily by salt water, are as a rule small rigid shrubs rarely exceeding 5 feet in height. The smallest plants are 1–2 feet high and are found in the most exposed situations. More sheltered plants growing along the banks of tidal rivers in brackish water may attain a height of 10–12 feet and are much more slender than the robust harbour forms. The habitat of this species is thus an area submerged by the rising tide under salt or brackish water.

Aegiceras majus is known as the "river mangrove". It extends north into Queensland, and is found also along the coasts of the old world tropics. Although it is frequently not as abundant as *Avicennia officinalis*, it can be distinguished easily from it at a distance by the bright green colour of the foliage.

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* The work was commenced when this writer held a Science Research Scholarship in Botany of the University of Sydney.

This genus is the only member of the Myrsinaceae which is present in the mangrove flora. Its characteristic flowers have been so fully described by systematists (Engler and Prantl, 1891) that any further description is unnecessary. The flowering period begins in early winter (May-June) and extends over several months. The phases immediately following fertilization proceed slowly, so that it is not till early December that endosperm formation is begun in the embryo sac. From this time development proceeds more rapidly, so that in late January the young embryo is sufficiently developed to emerge into the cavity of the ovary, and by March or April is about 3 cm. in length and ready to be shed from the tree. Owing to the fact that the period of fruit development extends over about a year, flower buds may be initiated before all the fruits of the previous season have been shed.

The smaller or "shrub" forms are found to produce larger fruits than the "tree" forms. The fruits are about ½–1 cm. longer, and proportionately thicker than those produced on the larger trees.

The Gynoecium.

The gynoecium is a flask-shaped structure, the style merging imperceptibly into the ovary. The latter has a conical loculus into which projects a free basal placenta (Text-fig. 1). Many of the cells in the wall of the ovary and in the style and placenta contain a substance, in the form of finely divided droplets, which, after fixation in formalin acetic alcohol, becomes yellow. This substance was tested by means of the standard microchemical methods (Haas and Hill, 1928, p. 79), and was found to give reactions for both proteinaceous and fatty material.

The stylar region of the gynoecium is studded with schizogenous glands (G, in Text-fig. 1). These may also be present occasionally in the ovary wall. Another feature of the style is the occurrence of young sclereids in whose walls are numerous simple pits. Here and there in the walls of the loculus, and particularly on the lower part of the expanded apex of the placenta, are glandular hairs. These hairs each have a globular head composed of a number of densely protoplasmic cells, borne on a stalk of 2–4 cells (Text-figs. 2*a*, 2*b*). Thus they differ appreciably from those which have been figured by Solereder from the foliage of the same species, and described by him (1908, pp. 610–611) "as especially peculiar in having their lateral walls fused with the wall of the depression and in the arrangement of the ray cells in surface view, which remind one of certain corals (*Fungia discus*)".

The placenta, arising at the base of the ovary, has, at the time of fertilization of the ovules, a short stalk which projects into an expanded apex. This in longitudinal section has the appearance of an arrow-head (Text-fig. 1). A trans-

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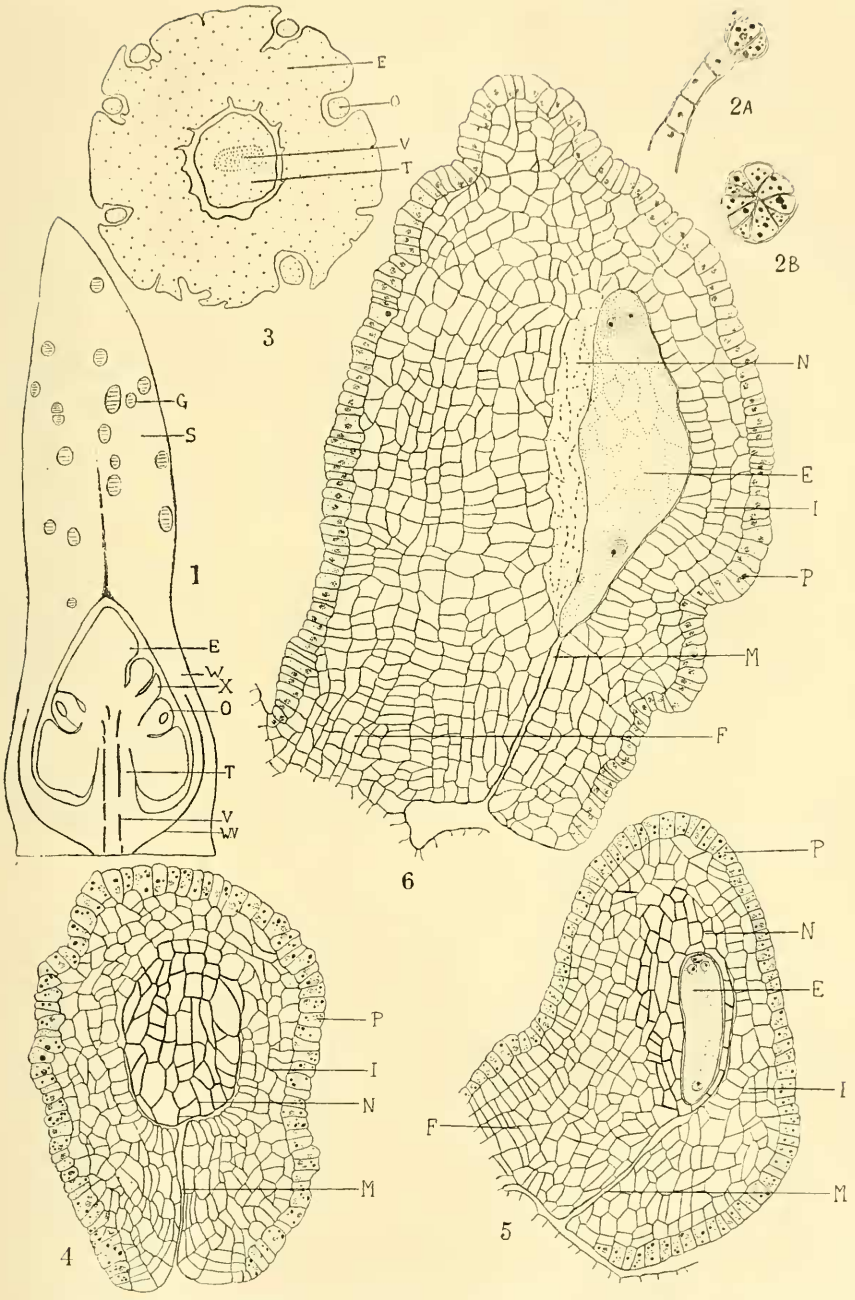
Text-fig. 2.—A, glandular hair from the wall of the ovary or placenta. B, surface view of the head of a glandular hair. × 250.

Text-fig. 3.—A transverse section of the expanded part of the placenta. E, lower part of the expanded apex of the placenta; O, ovule; T, stalk of placenta; V, vascular strand. × 49.

Text-fig. 4.—A tangential section of a young ovule. P, peripheral layer of the integument; I, integument; N, nucellus; M, micropyle. × 250.

Text-fig. 5.—A radial section of a young ovule at the time of fertilization. I, integument; P, peripheral layer of the integument; N, nucellus; E, embryo-sac; F, funicle; M, micropyle. × 250.

Text-fig. 6.—A radial section of an ovule enlarging after fertilization. I, integument; P, peripheral layer of integument; N, nucellus in process of being resorbed; E, embryo-sac; F, funicle; M, micropyle. × 250.



Text-fig. 1.—A longitudinal section of the ovary and part of the style. G, schizogenous gland; S, style; W, ovary wall; E, expanded part of the placenta; V, vascular strand of the placenta; WV, vascular strand of the ovary wall; X, projection of placenta tissue between ovules; O, ovule; T, stalk of placenta. × 19.

verse section passing through the upper region of the stalk (Text-fig. 3) shows the expanded apex as an apparently free tissue around the stalk. In this expanded apical region numerous ovules are embedded (Text-figs. 1, 3) so that there are projections of the placenta between them.

The Ovule at Fertilization.

Each ovule is anatropous and has a single massive integument (Text-fig. 5). Through this integument extends a long narrow micropyle which, as the funicle is very short, faces the placenta. The outermost layer of the integument is clearly differentiated from the inner layers, as the cytoplasm of the cells is denser, and globular food-bodies with the same staining properties as those mentioned previously in the placenta are clearly visible. The cells of the integument below the embryo-sac and those in the funicular region are arranged in fairly regular rows (Text-figs. 4, 5). As will be seen later, these cells are potentially meristematic.

There is no trace of vascular tissue in the funicle, so the ovule is without a conducting strand. The only vascular supply is found in the placenta, and here the centrally placed tracheids can be traced to a position almost level with the uppermost ovule (Text-fig. 1).

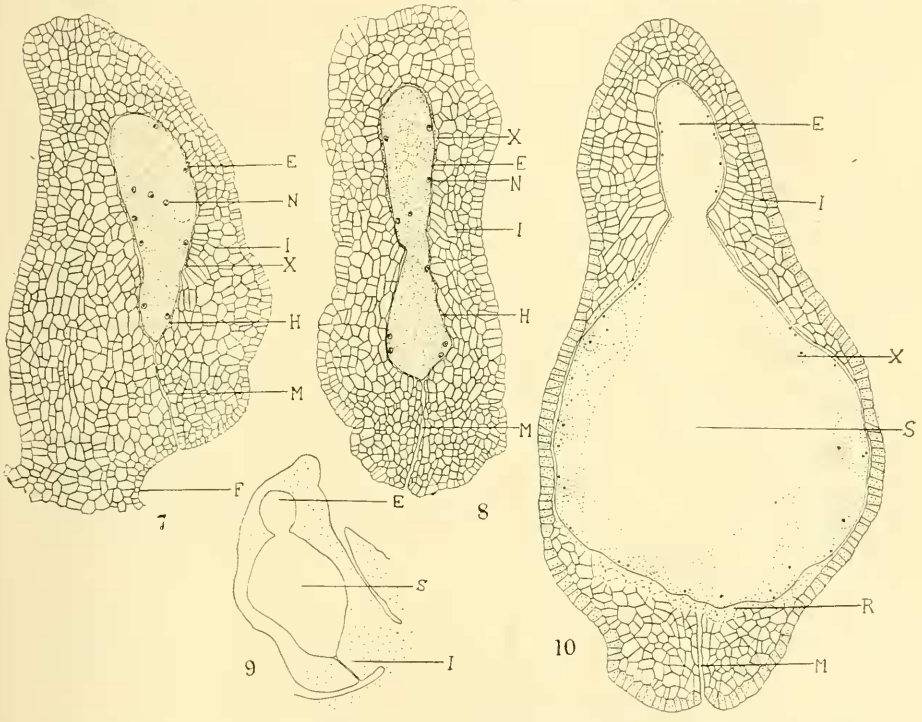
The nucellus is composed of cells which are larger and thicker-walled than those of the integument which invests it. Also the degree of fusion of the integument and the nucellus varies slightly. Text-figure 4 shows the nucellus free on both sides to about the median region of the embryo-sac, but frequently the nucellus on the outer side of the embryo sac is free to a point level with the base of the sac (Text-fig. 5).

The embryo-sac is elliptical in section and is excentrically placed in the nucellus, so that, on the side of the ovule remote from the funicle, there are only one or two rows of cells between it and the integument. The embryo sac previous to fertilization shows the eight nuclei typical of most dicotyledonous embryo-sacs.

Post-fertilization Stages.

Fertilization may occur in a number of ovules simultaneously in any one ovary. After fertilization, the ovule enlarges and the embryo sac increases in size at the expense of the nucellus. The nucellus on the side of the embryo-sac remote from the funicle, being thinner than that on the funicular side, becomes resorbed first. At one stage of its development, therefore, the embryo-sac appears to have nucellar tissue on the inner side only (Text-fig. 6). All traces of the nucellus are, however, soon lost and at this stage the embryo-sac is bounded only by the integument. The cells of the integument seem to offer a greater resistance to the growth of the embryo-sac than do the cells of the nucellus, as they are not affected by the resorbing action of the sac. This resistance causes the further extension of the embryo-sac, therefore, to be directed into the micropyle (Text-fig. 7). At the same time cell-division begins in the integument in the micropylar region, and proceeds with increasing rapidity. The embryo-sac continues to invade the micropyle, and so pushes down into this meristematic region (Text-figs. 7, 8). Successive divisions in this tissue result in the rapid elongation of the micropylar part of the integument, but the bulk of the tissue so formed is resorbed by the advancing embryo-sac. This resorption proceeds transversely as

well as longitudinally in the micropylar region of the ovule, so that the integument here, i.e., below the original embryo-sac, is gradually reduced to a single layer, except at the outer end of the micropyle. In this region it still remains massive and cell-divisions take place so that the tissue keeps pace with enlarging sac. Meristematic activity extends gradually from the region of the micropyle to the adjacent cells of the funicle, so an increase in size takes place here also. Remnants of the inner part of the integument persist for some time between the embryo-sac and the intact outer layers of the integument. At this stage in the development, the greater part of the ovule is occupied by the enlarged embryo-sac (Text-figs. 9, 10), whilst towards the apex of the ovule the outline of the original embryo-sac is retained by the integumental cells. At quite an early stage the cells of the



Text-fig. 7.—A radial section of a fertilized ovule showing the embryo-sac commencing to grow into the micropyle. I, integument; X, resorbed nucellus; E, embryo-sac; N, endosperm nuclei; H, haustorial part of the sac; M, micropyle; F, funicle. × 124.

Text-fig. 8.—A tangential section of a fertilized ovule at a slightly older stage than that shown in Text-fig. 7. I, integument; X, resorbed nucellus; E, embryo-sac; N, endosperm nuclei; H, haustorial part of the sac; M, micropyle. × 124.

Text-fig. 9.—A radial section of a fertilized ovule showing the embryo-sac much enlarged in the micropylar region. E, original position of embryo-sac; S, extension of the sac into the micropyle; I, integument. × 41.

Text-fig. 10.—A tangential section of a fertilized ovule at a stage similar to that shown in Text-fig. 9. I, integument; E, original position of embryo-sac; S, extension of the sac into the micropyle; X, endosperm nuclei; R, resorbed integumental tissue; M, micropyle. × 114.

funicle and the adjacent massive micropylar integument become filled with the characteristic proteinaceous food material.

During the enlargement of the embryo-sac the endosperm nucleus has undergone repeated divisions, giving rise to numerous small nuclei. The nuclei at first lie scattered throughout the embryo-sac (Text-figs. 7, 8), and can be clearly distinguished from the larger zygote nucleus, which is at the micropylar end of the sac. However, as the embryo-sac enlarges, the cytoplasm at the centre becomes very attenuated and the nuclei move to the periphery, at the same time increasing in size. This enlargement of the nuclei can be seen if Text-figures 7 and 8 (×124) are compared with Text-figure 10, which is drawn on a slightly smaller scale (×114) and yet shows obviously larger nuclei. At this stage the zygote nucleus can no longer be identified, but it apparently retains its position at the micropylar end of the advancing embryo-sac as the initial embryonal tissue makes its appearance in that position.

Several fertilized ovules may reach this stage of development, but one ovule, usually one situated towards the apex of the placenta, soon becomes dominant.

In the now single layer of the integument, divisions at right angles to the surface of the ovule take place so rapidly that this tissue falls into folds around the embryo-sac (Text-fig. 11A). At the same time cell wall formation begins at the periphery of the sac and advances inward till an endospermic tissue is formed. This tissue is composed of small cells at the periphery merging into larger central cells (Text-fig. 11B). The position of the original embryo-sac is still visible (Text-fig. 11A), and can be traced through the various stages of development till the time when the folds in the integument are straightened out by the growing embryo and endosperm. Meanwhile meristematic divisions continue in the funicle (F, in Text-fig. 11A) and in the adjacent part of the integument, and result in the widening of that area. The cells composing it are filled with reserve food material similar to that described in the integument of the young ovule. This material stains strongly with most reagents, e.g., safranin and haematoxylin, and is present in such quantities that after staining no detail of cell structure can be made out unless the sections are so destained that all other parts become almost colourless.

The embryo itself remains indistinguishable previous to the formation of endosperm tissue in the embryo-sac. It can be observed at the micropylar end of the embryo-sac, when endosperm cell formation is completed, and is at first quite close to the periphery of the sac (Text-fig. 12).

The small endosperm cells comprising the peripheral zone in the embryo-sac are more densely protoplasmic than the inner cells, and become meristematic

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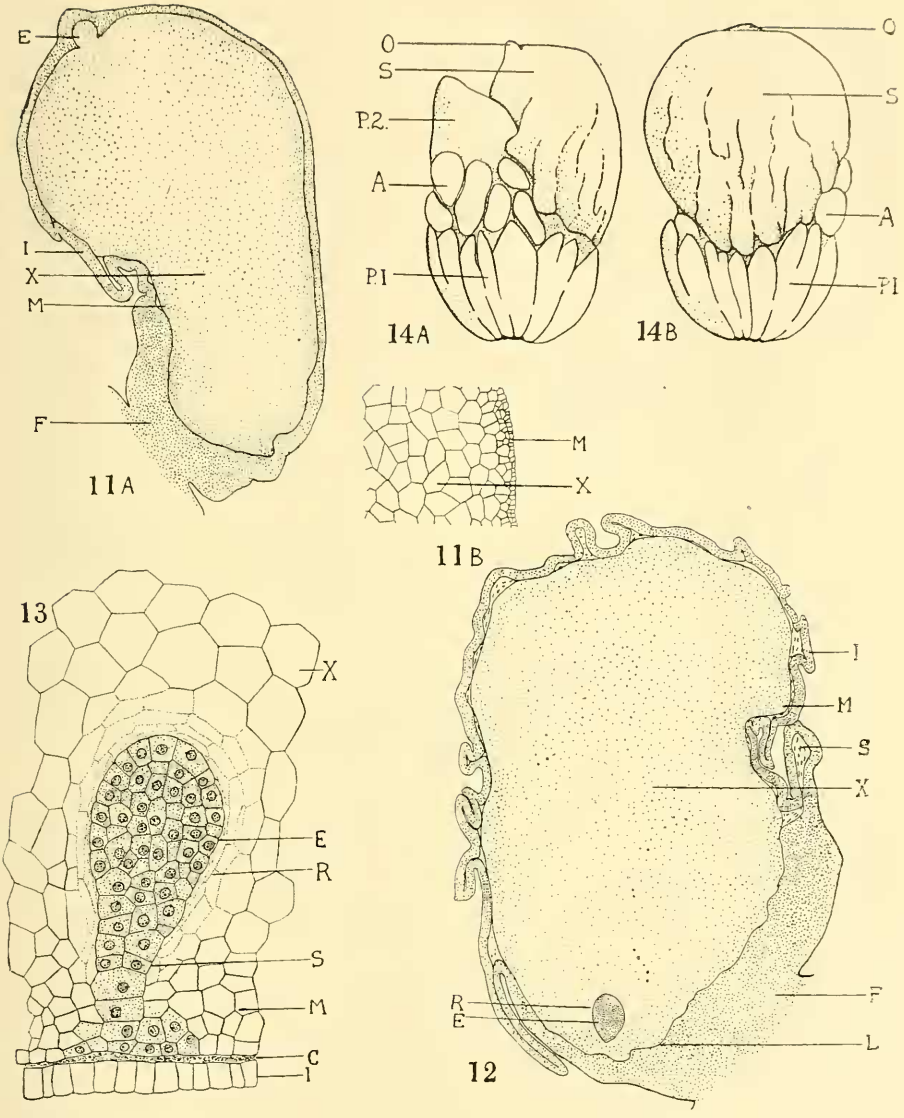
Text-fig. 13.—A young embryo embedded in the endosperm. X, inner primary endosperm; R, endosperm in process of being resorbed; E, embryo; S, suspensor; M, meristematic region of the endosperm; C, crushed inner layer of the integument; I, integument. × 280.

Text-fig. 14.—A. A view of the expanded portion of the placenta on which can be seen the aborted ovules (A) and a fertilized ovule at the stage shown in Text-fig. 12. O, position of original embryo sac; S, enlarged portion of the sac; P1, furrowed basal part of the expanded apex of the placenta; P2, tip of the placenta. × 10 approx.

B. A view of the placenta and developing seed taken at right angles to that shown in Text-fig. 14A. O, position of original embryo sac; S, enlarged part of the sac; A, aborted ovules; P1, furrowed basal part of the expanded apex of the placenta. × 10 approx.



Text-fig. 11.—A. A radial section of a fertilized ovule in which endosperm tissue has been formed. I, outer layer of the integument; E, original position of the embryo-sac; X, inner endosperm; M, outer endosperm; F, funicle. × 30.

B. Part of Text-fig. 11A shown in full cell detail. M, outer endosperm; X, inner endosperm. × 30.

Text-fig. 12.—A radial section of a developing seed showing the enlarging embryo and the differentiation of the endosperm into an outer and an inner region. I, outer layer of the integument; S, fragments of the inner part of the integument; M, meristematic region of the endosperm; X, inner primary endosperm; R, resorbed zone of the endosperm; E, embryo; F, funicle; L, endospermic lobes. × 30.

soon after their formation. By their activity new cells are formed, at first mainly in radial rows at right angles to the outer wall of the embryo-sac, but later cells are cut off in all directions in this peripheral zone. The new cells remain small, and thus a very distinct zone is formed around the margin of the endosperm (Text-fig. 13). Meristematic activity in this peripheral zone of the endosperm continues rapidly, with the result that the endosperm tissue increases in size. The result of this increase in size is particularly striking in the lower part of the ovule. Here the endosperm is in contact with the massive meristematic and food-filled tissue of the funicle and with the basal part of the integument in the micropylar region. When the outer endosperm becomes meristematic it also assumes a haustorial function. By continued cell division it forces its way into the massive tissue in a series of lobes (L, in Text-figs. 12, 15). These lobes in their mature condition have been fully described by Haberlandt. They increase in length and width, boring steadily into the adjacent tissues; sometimes branching into finger-like processes; sometimes twisted so that in section there appear to be isolated islands of meristematic haustorial endosperm in the tissue of the integument or funicle. It is evident that these endospermic folds resorb the integumental cells with which they come in contact, but there is very little evidence of crushing or alteration of tissue in advance of the folds. They are in very close contact with the cells of the funicular and integumental tissue they invade. In contrast with this, traces of crushed cells are often seen between the endosperm and integument on the flanks and top of the endosperm.

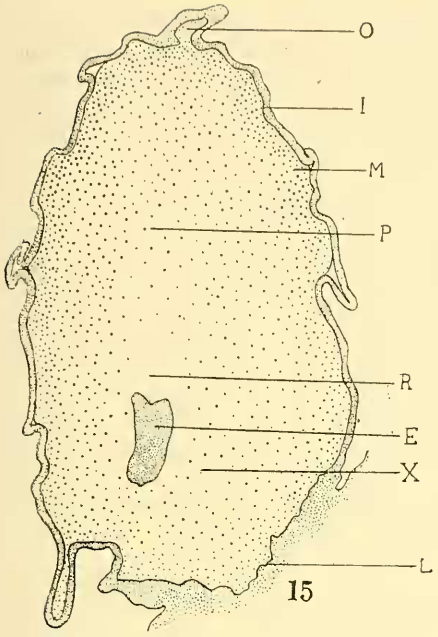
In spite of the haustorial action of the endospermic folds, the thickness of the invaded tissue of the integument and funicle is maintained and even increased by continued cell division in all planes. Cell division in the rest of the peripheral zone of the endosperm, i.e., in the upper part of the seed, away from the micropyle and funicle, is not so marked as in the lower part. It results in a general increase in size of the developing seed.

Meristematic activity in the endosperm is practically limited to the outer 6–7 rows of cells, so that a secondary tissue is formed behind the meristematic region by its continued advance. The cells of this tissue remain small and thin-walled and constitute what may be referred to as the secondary endosperm, in contrast with the large-celled primary endosperm filling the centre of the embryo-sac.

At the same time the remaining single row of cells constituting the major part of the integument continues to divide rapidly in a plane at right angles to the surface of the ovule, so that it becomes more and more wrinkled over the surface of the endosperm, in spite of the continued growth of that tissue (Text-fig. 12).

The embryo rapidly increases in size, and by the time meristematic activity is established in the peripheral endosperm, the divisions of the fertilized egg have resulted in the formation of an embryo such as is shown in Text-figure 13.

A study of the seed at this stage of development (i.e., at the stage indicated in Text-fig. 12) shows that the much enlarged embryo-sac is not spherical, but is rather spheroidal in shape. Looking at the seed in radial view, it appears fairly narrow, lying obliquely on the placenta, with the original embryo-sac visible at the apex (Text-fig. 14A), while from another aspect at right angles to the first (Text-fig. 14B), it appears much wider and the heavy folding of the integument is more clearly shown. The growth of the seed is already pushing the apex of the



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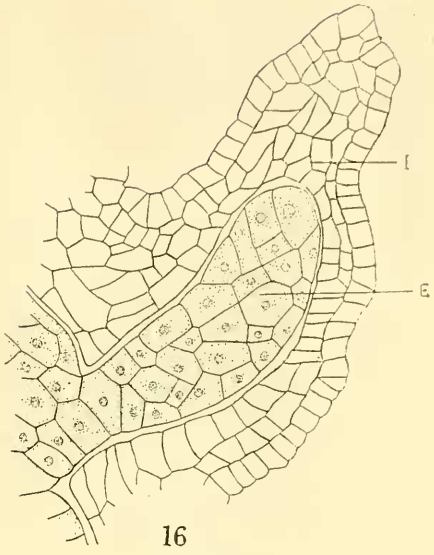

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Text-fig. 15.—A radial section of an older seed than that shown in Text-fig. 12. O, position of the original embryo-sac; I, integument; M, meristematic region of the endosperm; E, embryo; X, endosperm which has been attacked by enzymes; L, endospermic lobes; R, primary endosperm in process of being resorbed; P, unaltered primary endosperm. × 30.

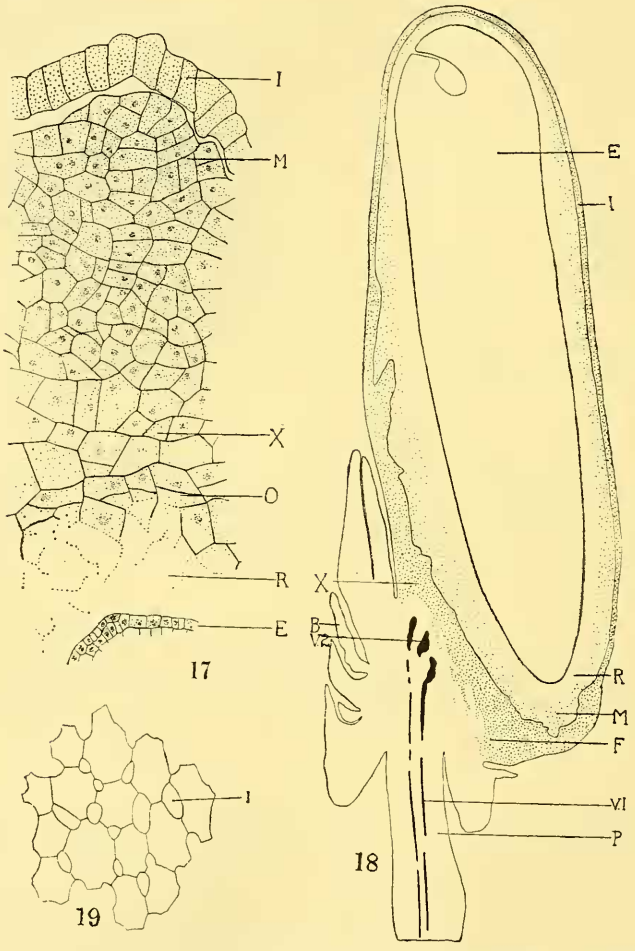
Text-fig. 16.—The original embryo-sac of Text-fig. 15 shown in detail. I, integument; E, endosperm. × 262.

arrow-shaped head of the placenta to one side of the loculus of the ovary. The lobing and furrowing characteristic of the lower part of the apex of the placenta is also clearly shown in Text-figures 14A and 14B. In the median region of the expanded apex of the placenta (Text-fig. 14A) many aborted ovules can be seen.

The Growth of the Embryo.

The embryo increases in size at the expense of the neighbouring endosperm cells. These endosperm cells are first altered by the action of enzymes secreted by the embryo, since, although they are quite undeformed, cells at a considerable distance from the embryo show evidence of alteration by their reaction to stains. The altered endosperm cells are then crushed by the growth of the embryo and their contents are resorbed. Around the growing embryo, therefore, there are a number of zones. Firstly, adjacent to the embryo, are the structureless remains of the cells whose contents are being resorbed, cell walls are disintegrating and nuclei are no longer visible (R, in Text-fig. 17); further out is a zone of cells whose contents appear homogeneous, due to enzyme action, and which are slightly deformed by the pressure of the growing embryo (U, in Text-fig. 17); further out still, is the zone of undeformed cells which are in the process of alteration by enzyme action (X, in Text-fig. 17), and this grades into the unaltered endosperm. The altered zones are readily distinguished in stained sections, since they stain

practically uniformly with dyes such as anilin blue and Delafield's haematoxylin. At the periphery of the endosperm the meristematic zone of densely protoplasmic cells is evident (M, in Text-fig. 17), while between this zone and the integument, crushed fragments of the cells which composed the inner layers of the integument can sometimes be made out.



Text-fig. 17.—Part of the developing seed shown in Text-fig. 15 shown in full cell detail. I, integument; M, meristematic region of the endosperm; X, endosperm which has been attacked by enzymes; O, slightly deformed endosperm; R, resorbed endosperm; E, embryo. × 250.

Text-fig. 18.—A radial section of a seed and the placenta showing a much enlarged embryo which extends the full length of the seed. I, integument; E, embryo; R, inner endosperm in the process of resorption; M, outer meristematic and haustorial endosperm; F, funicle; B, aborted ovules; P, placenta; V1, conducting tissue; V2, vascular tissue just below the funicle. × 19.

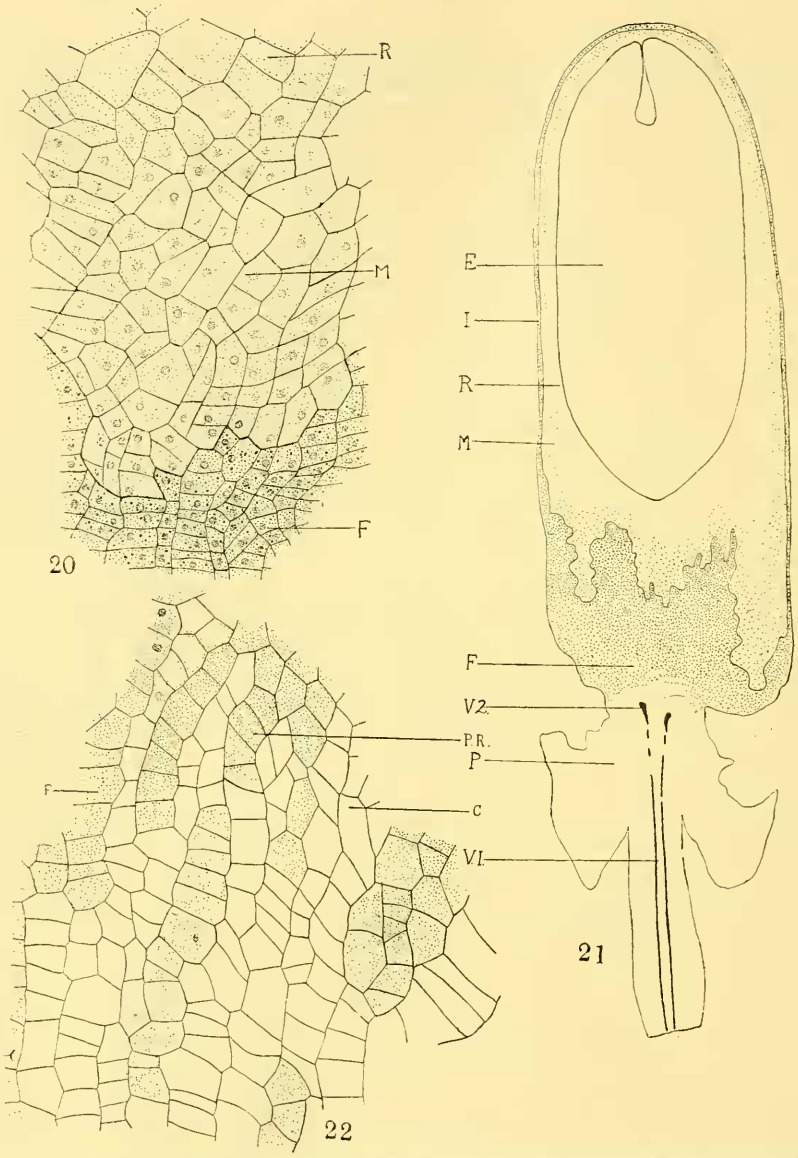
Text-fig. 19.—A small portion of a tangential section of the stalk of the placenta showing intercellular spaces (I). × 267.

The young embryo grows rapidly, and by the time it is 0·4 mm. in length it begins to show signs of the development of cotyledons (Text-fig. 15). Text-figure 15 also shows the original position of the embryo-sac at O, at the apex of the ovule. This part is shown in detail in Text-figure 16. The integument (I) is thicker here than on the flanks of the ovule, and the position of the original embryo-sac is filled with a small-celled prolongation of the endospermic tissue (E).

As the embryo increases in size the primary endosperm becomes wholly resorbed and the secondary endosperm is next attacked. The subsequent growth of the embryo is very rapid, and to accommodate it and provide the necessary food material, the growth of the haustorial and meristematic endosperm is also very rapid, the haustorial lobes becoming very much increased in size and complexity. Continued divisions of the cells of the massive region of the funicle and integument enable this tissue to maintain its dimensions and so still provide the food supply for the haustorial and primary endosperm and the embryo. Divisions in the meristematic endosperm along the flanks and over the apex of the ovule enable it to keep pace with the rapidly enlarging embryo, but during this process it becomes very thin (Text-figs. 18, 21). This enlargement of the embryo and endosperm causes the folds in the integument to be stretched out (Text-fig. 18), so that this layer soon fits very tightly over the surface of the endosperm.

The haustorial folds of the endosperm are now very numerous. In most cases there are a relatively few major haustoria such as are shown in Text-figure 21, and from these, numerous small subsidiary haustoria develop into the integument and funicle. At the stage shown in Text-figure 21, these haustoria have rather blunt apices consisting of a number of small cells. Occasionally one finds narrow haustoria one or two cells wide forcing their way into the funicle. The major folds seem to be developed chiefly vertically in a plane tangential to the placenta, since they are best seen in tangential longitudinal sections. Transverse sections taken just below the vascular strand of the placenta (about X, in Text-fig. 18) show the subsidiary folds excellently, and the position of a major fold may be indicated by a wide shallow extension of the endosperm. Radial sections frequently miss the major folds altogether, and show only the smaller ones (Text-fig. 18). When the embryo is nearing maturity and has emerged into the cavity of the ovary, the cells at the ends of the haustoria tend to grow out separately for short distances. This development is specially well seen in transverse sections taken just above the vascular strand of the placenta, and has been very adequately described by Haberlandt. In transverse sections which include the vascular strand of the placenta, individual haustorial lobes are less numerous and less deeply penetrating than in sections taken higher up. The endosperm tissue encroaches on this part of the funicle in an almost unbroken front. In contrast to this Haberlandt found that in the ovules examined by him the haustorial lobes were more pronounced here than elsewhere.

The contact between the cells of the endospermic folds and the cells of the massive tissue they invade is, as was described before, very close, so that without a knowledge of the previous history of the two tissues, one would have difficulty in distinguishing them as of separate origin. In Text-figure 20 a small section of this region is shown in detail; at F, are the thin-walled dividing cells of the funicle, which are filled with darkly staining reserve food and are arranged in more or less definite rows; at M, are the cells of the haustorial endosperm, also



Text-fig. 20.—Part of a radial section of a seed showing the full cell detail of the meristematic region of the funicle (F). M, haustorial endosperm; R, inner endosperm. × 250.

Text-fig. 21.—A longitudinal section passing through the placenta and tangentially through the upper part of the embryo. The embryo is slightly older than that shown in Text-fig. 18. E, embryo; I, integument; R, inner endosperm undergoing resorption; M, haustorial endosperm; F, funicle; V1, vascular tissue of the placenta; V2, vascular tissue just below the funicle; P, placenta. × 19.

Text-fig. 22.—A detailed study of the part of the placenta adjoining the funicle, showing rows of cells filled with proteinaceous material. F, funicular cells; PR, cells of the placenta filled with proteinaceous material; C, clear cells of the placenta. × 250.

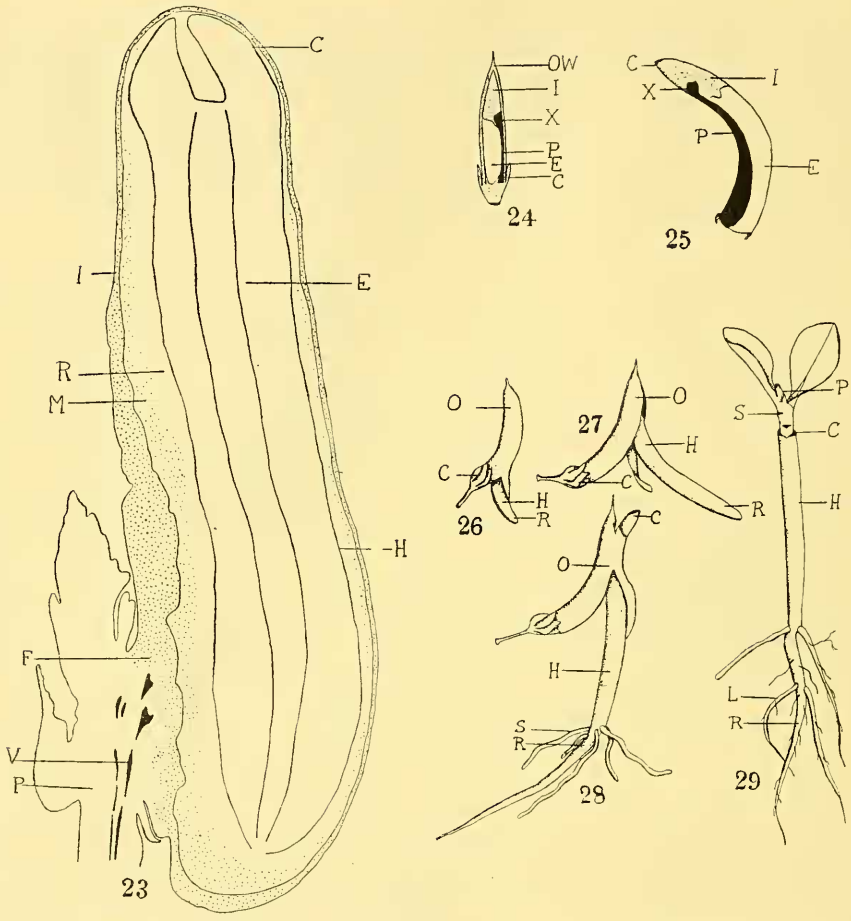
in fairly regular rows, but distinct from the rows of the funicular cells they invade. These cells are thin walled and densely protoplasmic, but stain less darkly than the cells of the funicle, since they have no reserve food. Behind this actively meristematic zone is the region where divisions have ceased and upon which the growing embryo is encroaching. Haberlandt (1895) has given a detailed account of the embryo at about this stage of development, but since the embryos examined by the present writers differ slightly from those described by him, Haberlandt's observations will be discussed later, and a complete description is given here of the facts as observed by us.

During the period of enlargement of the embryo, the stalk of the placenta increases in length (cf. Text-figs. 18 and 21). This process seems to be simply one of cell expansion, the individual cells becoming larger and broader, and air spaces appearing between them (Text-fig. 19).

To accommodate the increase in size of the funicle, meristematic divisions take place in the adjacent part of the placenta. New elements are added to the vascular strand which extends towards the funicle but never into it, or into the integument. It is obvious, therefore, that food material for the growing embryo must first pass across the small cells of the funicle and thence be absorbed by the haustorial endosperm. The cells of the funicle are small, fairly thin walled and stain darkly because of the presence of food material within them. The cells of the placenta are similar in size and shape, but food material is stored only in a few scattered cells, so that the tissue as a whole does not stain darkly. The two tissues are, therefore, fairly sharply differentiated from one another. Where they merge it is often found that rows of cells filled with proteinaceous food material and rows of clear cells alternate over a short area (Text-fig. 22).

By the time the embryo has reached a length of 5 mm. (Text-fig. 18) the cotyledons are fully differentiated. They are short and fleshy, and in longitudinal section appear triangular. Occasionally they are slightly twisted around one another. The meristem of the radicle is also distinguishable at this stage, but remains dormant and undifferentiated until the embryo begins to germinate. The embryo by this time has become a deep green, due to the presence of chlorophyll in the cells of the cotyledons and the cortex of the hypocotyl. In older specimens multicellular hairs, as described by Haberlandt (1895), are present around the young radicle. The plumule remains as an undifferentiated meristem between the cotyledons until the young plant becomes established in the soil.

Up till the stage shown in Text-figures 18 and 21, increase in size is due to cell divisions throughout the whole embryo, but now growth becomes practically confined to the lower part of the hypocotyl. The radicle, therefore, becomes gradually pushed down to a position below the apex of the placenta (Text-fig. 23). The integument and endosperm keep pace with this extension in length by continued cell division. But eventually the elongation of the hypocotyl causes the radicle to break through the integument at the lower end of the ovule so that it emerges into the cavity of the ovary. At the same time the stalk of the placenta continues to elongate, so that the integument and the endosperm remain as a close fitting cap over the cotyledons and upper part of the hypocotyl. This condition is shown in Text-figures 24 and 25 (see also Haberlandt, 1895, Text-fig. 8). The embryo by this time completely fills the ovary, and the placenta, endosperm and integument are crushed very closely against it. As the embryo approaches its mature size (Text-fig. 25) it becomes curved, with the placenta along the concave side. This bending appears to be due to the fact that the



Text-fig. 23.—A radial section of a seed older than that shown in Text-fig. 18. The extension of the hypocotyl has carried the base of the embryo below the level of the apex of the placenta. E, embryo; C, cotyledons; I, integument; R, inner resorbed endosperm; M, haustorial endosperm; H, hypocotyl; F, funicle; P, placenta; V, vascular tissue. × 19.

Text-fig. 24.—A diagram showing the young embryo within the ovary. Part of the ovary wall is removed. OW, ovary wall; I, integument; X, upper part of the placenta and aborted ovules; P, placenta; E, embryo; C, persistent calyx. × 0·7.

Text-fig. 25.—An embryo removed from the fruit. C, tip of the cotyledons; E, embryo; I, integument; X, upper part of the placenta and aborted ovules; P, placenta. × 0·95.

Text-fig. 26.—An embryo emerging from the fruit. R, radicle; H, hypocotyl; C, persistent calyx; O, ovary wall. × 0·6.

Text-fig. 27.—A later stage in the emergence of the embryo from the ovary wall. R, radicle; H, hypocotyl; O, ovary wall; C, persistent calyx. × 0·76.

Text-fig. 28.—An older embryo which has developed secondary roots (S). The cotyledons are emerging from the ovary wall (O). R, radicle; C, cotyledons; H, hypocotyl. × 0·76.

Text-fig. 29.—A well established seedling. P, shoot; S, leaf scar; C, cotyledons; H, hypocotyl; R, main root; L, lateral roots. × 0·48.

stalk of the placenta is unable to extend as rapidly as does the hypocotyl, and consequently causes the embryo to arch away from it.

The embryo has by this stage become packed with starch, which is stored as compound grains throughout the pith and cortex of the hypocotyl. It also contains a considerable amount of proteinaceous reserve food material similar to that described in the funicle of the young ovule. A section taken through the funicle and endosperm at this stage shows that the endosperm is practically all resorbed, and that the walls of the invading haustoria become thickened as described by Haberlandt (1895), and the apices of the haustorial lobes tend to branch into the funicle in finger-like processes one cell wide. The thickening of the walls causes the cells to draw away at the corners, leaving air spaces, till finally they become almost separated from each other and from the funicular cells. When the embryo is mature, the endosperm and integument are almost completely dried up, and remain fitting like a cap over the cotyledonary end of the embryo. In the specimens examined by us the funicle never became wholly depleted of its characteristic proteinaceous reserve food.

An account of the emergence and establishment of the seedling has already been given by Collins (1921) in connection with her work on the mangrove vegetation of the Sydney district (N.S.W.), but for the sake of completeness a brief description of the stages in the development of the embryo up to the time of its establishment in the soil will be included here.

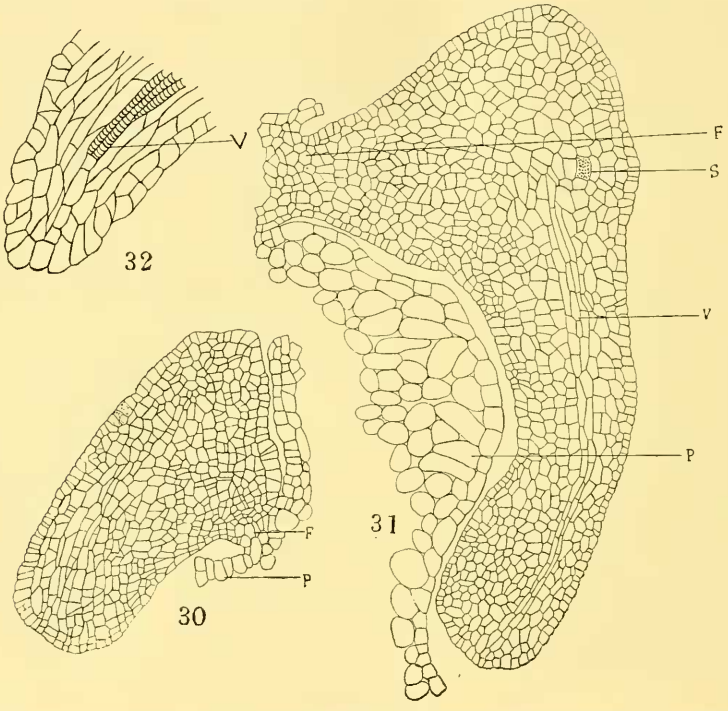
Rapid growth continues in the hypocotyl until the embryo is nearly 3 cm. in length. At about this time the fruit falls from the parent plant, but no great check occurs in the process of elongation of the embryo. It soon bursts through the wall of the ovary (Text-figs. 26, 27) and the radicle end of the embryo emerges. If it should have fallen in a suitable place the radicle quickly grows down into the mud and secondary roots are formed which securely anchor the young plant (Text-fig. 28). The cotyledons then emerge from the ovary wall, casting off the integument, and the plumule grows up between them and expands (Text-fig. 29). The reserve food in the hypocotyl begins to disappear as the young plant increases in size, but a considerable amount is still present at the stage shown in Text-figure 29.

"Appendages."

Curious structures which occupy the position of ovules on the placenta and at first resemble them in certain respects are occasionally met with in sections of ovaries. In a young ovary they present an appearance such as is shown in Text-figure 30. Each has a short funicle-like stalk (F) and is somewhat elongated downwards. The tissue composing it, however, is quite homogeneous, there being no indication of integument or embryo-sac, so that the structure cannot be regarded as an ovule, unless possibly as an abnormal development of nucellar tissue. The outermost layer of cells contains proteinaceous material and stains deeply, resembling the peripheral layer of the integument of normal ovules.

As the ovary increases in size and the fertilized ovules develop, these "appendages" elongate rapidly downwards along the furrows of the expanded part of the placenta. As they grow they become slightly twisted, so that it is impossible to obtain a complete view of a fully developed "appendage" in any one section. That shown in Text-figure 31 is by no means a large specimen. The central cells of the elongated part of this structure become long and narrow, and finally develop close spiral thickenings on their walls, so that a full grown

"appendage" appeared to have a well developed vascular strand down the centre. These spiral elements reach almost to the tip of the structure, as shown in Text-figure 32, but they extend back through the "appendage" only as far as will bring them into close proximity with a schizogenous gland, which occurs in a position approximately analogous to that of the embryo-sac in the normal ovule. The "vascular tissue", therefore, does not pass through the funicle-like stalk and so has no connection with the conducting elements of the placenta.



Text-fig. 30.—A young "appendage". F, funicle-like stalk; P, placenta. × 148.

Text-fig. 31.—A later stage in the development of the structure shown in Text-fig. 30. F, funicle-like stalk; S, schizogenous gland; V, elongated cells which later develop spiral thickenings; P, placenta. × 148.

Text-fig. 32.—The tip of an "appendage" older than that shown in Text-fig. 31. V, cells with spiral thickenings. × 250.

The presence of one or more schizogenous glands (S, in Text-fig. 31), similar to those occurring in the ovary wall, is remarkable since no other glands develop at any time in the placenta or normal ovules.

Discussion.

1. *Comparison with Haberlandt's description.*

In his description of the embryology of *Aegiceras majus*, Haberlandt (1895) states that the mature fruit attained a total length of 7 cm., whereas in the Sydney district the mature fruits do not as a rule exceed 3·5 cm. in length, and the "river" forms have even shorter, more slender fruits. As Sydney is almost

the extreme southern limit of the distribution of *Aegiceras*, it is probable that the plants here are much less robust than those growing in the tropics, and that the fruits are correspondingly smaller. This difference in size is probably responsible for some of the discrepancies between Haberlandt's description and that of the present writers.

Haberlandt states that in ovules whose embryos were 7 mm. long the "Schleimendosperm", i.e., the inner endosperm which is in the process of being resorbed, attains a thickness of 0·2 to 0·3 mm. opposite the placenta. In ovules of similar size examined by the writers, the width of this endosperm was never more than 0·1 mm. Since, however, the mature fruits of the form examined by Haberlandt were about twice the size of those examined during the present investigation, Haberlandt's embryo 0·7 mm. in length was probably at an earlier developmental stage than an embryo of similar size collected near Sydney. Also, it might be reasonable to expect a more massive endosperm in the tropical form than in the southern form.

Haberlandt gives the following description of the inner endosperm (p. 107): "Die innere, dem Embryo anliegende Endospermschicht besteht in jüngeren Samen, deren Embryonen die Mikropyle noch nicht durchbrochen haben, aus ziemlich dickwandigen, mit zahlreichen grossen Tüpfeln versehenen Zellen, welche in ihrem Aussehen etwa den Endospermzellen von *Lupinus* gleichen; . . . Später nimmt diese endospermschicht den Charakter der Schleimendosperme an." This by no means corresponds with the condition observed by the present writers. In this investigation the inner endosperm is found to be uniformly thin walled at all stages of the development of the embryo, and never at any time resembles the endosperm of *Lupinus*, and pits, if present, are quite inconspicuous.

He also suggests that the "Schleimendosperm" may be of the nature of a water reservoir. But the observations here recorded show conclusively that this endosperm is nothing other than endospermic tissue slightly altered by the action of enzymes secreted by the growing embryo, and in the process of being resorbed. Both primary and secondarily formed endosperm are used up in this fashion.

In the Buitenzorg specimens the cells of the haustorial endosperm are shown by Haberlandt (Text-figs. 1–5) as being roughly half the size of the cells of the placental (i.e., funicular, in the present terminology) tissue they invade. In the specimens here described, it was found that the cells of the haustorial endosperm were little, if any, smaller than the adjacent cells of the funicle. Another difference between the Sydney material and that examined by Haberlandt is that in the former the cells of the funicle and massive micropylar part of the integument are filled with a proteinaceous reserve food material. In unstained sections this appears as a dense yellowish mass completely filling the cells. It also absorbs dyes very readily and holds them most tenaciously. This reserve food material appears at a very early stage in the development of the ovule; it is not at any time completely exhausted by the embryo, so that even in mature ovules a proteinaceous residue still remains. Haberlandt's figures (Figs. 2–5) and description of a section through the embryo of a fruit 17 mm. or more long, show placental (i.e., funicular, in the present terminology) cells as large brown-walled cells, poor in protoplasm; the presence of proteinaceous material within them is not mentioned. In the present investigation it was found that the walls of the funicular cells remain relatively thin, so long as the associated haustoria remain functional.

2. *The nutrition of the embryo.*

In the Sydney district the time elapsing between flowering and maturation of the embryo is 9–10 months. Growth is slowest during the early developmental stages up till the time of formation of cellular endosperm. This was found to take about 7 months. As soon as the endospermic haustoria have been initiated, growth becomes relatively much more rapid. From the foregoing description of the development of the embryo, it would seem reasonable to attribute this slow initial growth to the fact that food material for the developing embryo must pass through a zone of meristematic cells. In such a case food material would be obtained with greater difficulty than if the funicle had been equipped with a vascular system, and consequently the growth would be comparatively slow. Once the endospermic haustoria are formed, however, the obtaining of food material would be much facilitated; consequently the embryo would increase rapidly in size.

3. *Comparison with* Avicennia officinalis.

As Haberlandt pointed out, there is a general resemblance between the embryos of *Avicennia* and *Aegiceras* in that, in both cases, there has been a modification of the endospermic tissue to meet the requirements of a large viviparous embryo. In *Avicennia* (Treub, 1883), a single endosperm cell, much enlarged and branched, functions as the haustorium. In *Aegiceras*, lobes of endosperm grow into the funicle tissue and function as haustoria. But there is a further resemblance between the two, which a study of the youngest stages of development of the embryo of *Aegiceras* has brought to light. In *Avicennia officinalis* the nucellus is quickly resorbed and the endosperm and embryo-sac pass gradually out through the micropyle, with the exception of a single endosperm cell which remains within the integument and grows and branches, functioning as a haustorium. In *Aegiceras majus* there is a similar tendency. The nucellus is resorbed at an early stage and the embryo-sac commences to enlarge into the micropyle. It never succeeds in growing completely out, however, since cell division in the integument enables that tissue to keep pace with the growth of the embryo-sac, until the embryo is almost mature.

4. "*Appendages.*"

The function of the elongated "appendages" found in some ovaries remains obscure. They bear no resemblance to normal ovules, except in the presence of a funicle-like stalk and the size of their cells.

Summary.

1. *Aegiceras majus* Gaertn., a mangrove occurring in the Sydney district, is characterized by vivipary.

2. The carpels enclose a conical loculus in which develops the basal placenta. This placenta is expanded at the apex into an arrow-shaped head in which numerous anatropous ovules are embedded.

3. Each ovule has a massive integument. The cells in the micropylar region are potentially meristematic. The funicle is very short, and is composed of cells which are also potentially meristematic. No vascular tissue passes across the funicle to the integument.

4. The embryo-sac is elongated and is excentrically placed in the nucellus.

5. After fertilization the embryo-sac enlarges, crushing and resorbing the nucellus. It then commences to grow down into the micropyle.

6. The integument cells in this region divide rapidly radially and longitudinally. The embryo-sac continues to increase in size, absorbing the integument tissue as rapidly as it is formed.

7. Meanwhile the endosperm nucleus has divided many times so that numerous nuclei are found at the periphery of the enlarged embryo-sac.

8. The cells of the integument continue to divide, the successive divisions being so rapid that numerous folds are formed. At about this time cell formation commences at the periphery of the sac and advances inwards so that an extensive endosperm tissue is laid down. The outline of the original embryo-sac is still maintained by the cells of the integument.

9. The fertilized egg divides to form a short suspensor and embryonal tissue at the micropylar end of the sac.

10. The peripheral cells of the endosperm then become meristematic, causing general enlargement of the endosperm, until it completely fills and straightens out the integument.

11. Meanwhile the funicle has become widened by cell division, and the lower part of the endosperm grows into it in extensive folds. These folds assume a haustorial function.

12. The embryo grows rapidly, and the endosperm and integument increase in size by cell division, so that the embryo remains for a considerable period enclosed within them.

13. Finally the embryo grows through the endosperm and integument at the lower end, and the radicle and lower part of the hypocotyl emerge into the cavity of the ovary.

14. At the same time the placenta increases in length, enabling the relative position of the upper part of the embryo to remain unchanged with regard to the placental tissue, so that food continues to pass to the growing embryo.

15. The fruit is then shed from the tree. Under favourable conditions the hypocotyl continues to elongate, causing the radicle to break through the wall of the ovary and emerge.

16. Secondary roots are produced and the plant is established in the mud. The cotyledons break through the ovary wall and the plumule grows up between them.

17. Certain abnormal "appendages" which may occur on the placenta in the position of ovules are described.

18. Haberlandt's description of the endosperm of *Aegiceras majus* is discussed. A comparison is drawn between the mode of development of the embryo in *Aegiceras majus* and in *Avicennia officinalis*.

The authors wish to express their thanks to Professor T. G. B. Osborn, of the Department of Botany, Sydney University, for suggestions and kindly criticism throughout the course of this work.

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THE AUSTRALIAN SPECIES OF *GRAPHOMYIA* (DIPTERA, MUSCIDAE).

By I. M. Mackerras, B.Sc., M.B., Ch.M.,
Commonwealth Council for Scientific and Industrial Research,
Canberra, F.C.T.

[Read 30th November, 1932.]

The genus *Graphomyia* R.-D. includes a fairly compact assemblage of flies, which for the most part have a superficial resemblance to large and ornate species of *Musca*. Like that genus, the greatest number and variety of species are found in the tropical parts of the old world, nine of the twenty-two species at present recognized being African and nine Oriental. The genotype, *G. maculata* Scop., is the only one which is widespread, being recorded from Europe and Africa and represented by a subspecies in the Oriental and Australasian regions. One species has been described from Mexico and one from Chile.

In the Oriental region, one species, *G. luteicornis* Senior-White, occurs in Ceylon; one, *G. maculata rufitibia* Stein, in Formosa; two, *G. fasciyera* Stein and *G. atripes* Malloch, in Sumatra; four, *G. maculata rufitibia* Stein, *G. mellina* Stein, *G. vittata* Stein and *G. adumbrata* Wied., in Java; and two, *G. confluens* Stein and *G. rufiventris* Stein, in Ceram. No species, so far as I can discover, has been recorded from India. The distribution indicated is suggestive, but practically all the species are rare and it would not be safe to discuss the zoogeography of the group until a great deal more collecting has been done in various parts of the region.

Only one species, *G. eximia* Stein from New Guinea (*Nova Guinea*, xiii, Zool., 2, 1919, 199), has been described from the Australasian region. Malloch (these Proceedings, 1 (2), 1925, 46) states that he has seen a species from Australia, but gives no details concerning it. I have only seen the two species which are described below. It is clear that the genus entered Australia from the north, probably at a relatively recent date, and that it represents the "tail" of a widespread Malayan distribution.

Nothing is known of the biology of any of the species except *G. maculata* Scop., the larvae of which are saprophagous.

I am indebted to Mr. F. H. Taylor for the loan of the specimens recorded below from his own and the Ferguson collection, and to Dr. A. B. Walkom and Mr. A. Musgrave for copies of descriptions which would otherwise not have been available to me.

Genus Graphomyia R.-D.

The Australian species of *Graphomyia* may be distinguished from other Muscinae by the following characters: propleura, prosternum and mesopleura bare; hypopleura with fine hairs on upper part below and anterior to spiracle; acrostichal bristles reduced to one pre-scutellar pair; cell R_5 rather widely open and vein M_1 (distal section of M_{1+2}) with a rounded bend.

Graphomyia maculata rufitibia Stein.

The specimens before me agree very well with Stein's description of *G. rufitibia* Stein from Formosa and Java (*Ann. Mus. Nat. Hung.*, xvi, 1918, 147).

He had at first considered that his specimens represented a variation of *G. maculata* Scop., but later, because of their constantly slightly smaller size and more definite markings, he described them as a distinct species. There is a female of the typical form of *G. maculata* Scop. from France in the Ferguson collection. It does differ from the Australian specimens, but so slightly that I can only regard them as worthy of subspecific rank. I have reduced Stein's name accordingly. I give a description, based on Australian material, to facilitate identification by Australian workers.

♂. Eyes separated by about one-eighth of head-width, densely covered with short fine pale-brown hairs; frons black, parafrontals and parafacials shining silvery-white.

Thorax greyish-white, with an elongate-triangular median black stripe from suture to scutellum, a pair of slightly divergent narrow submedian black stripes extending from anterior end of thorax to a point mid-way between suture and scutellum, and a broad pair of sublateral black stripes which do not extend quite to anterior margin or to scutellum, and are narrowly interrupted at suture. Scutellum greyish-white, with a broad median black patch and black side margins. Pleurae covered with ashy tomentum, bristles and hairs black.

Abdomen covered with silvery tomentum. First visible tergite with a median black vitta which does not quite reach the hind margin, and with a broad sickle-shaped brown patch on each side extending along lateral and posterior margins but not reaching mid line. Second tergite with a narrow incomplete median black vitta and large sublateral brown patches. Third tergite with a narrower median black vitta, with a large black spot near the posterior edge on each side, and with brown sublateral patches extending the full length of tergite. Fourth tergite mostly covered with silvery tomentum, but with irregular submedian brown patches. Ventral aspect of tergites silvery and pale yellow; sternites silvery.

Legs with femora black, covered with ashy tomentum; tibiae brown; tarsi black. Wings faintly yellowish, veins bright brown; bristles on R_{1+5} extending from base two-thirds of way to r–m above and one-third of way to r–m below. Length, 7·5 to 8 mm.

♀. Eyes finely and rather scantily pubescent, separated by about one-third of head-width; frons black, with a median grey stripe commencing at the sides of the ocellar triangle and narrowing towards the antennae. Parafrontals and parafacials creamy-white. Thorax more yellowish than in ♂, but otherwise similar.

Abdomen covered with greyish-white tomentum and bearing the following brownish-black marks: first visible tergite with a median triangular vitta not reaching the apex of the segment, and with broad sickle-shaped patches extending from the lateral margins almost to mid line close to but not touching the posterior border; second tergite with a median lozenge-shaped vitta and irregular submedian and sublateral stripes extending from the anterior to the posterior border of the segment; third tergite with a narrow median vitta, submedian posterior black spots, and broad irregular sublateral patches extending the length of the segment; fourth tergite with small irregular patches. Insertion of abdominal hairs marked by small brownish-black spots, which are more conspicuous than in ♂. Venter, legs and wings as in ♂. Length, 7·0 to 7·5 mm.

One ♀ from Broadwater, New South Wales, differs from the others in the more strongly contrasted abdominal markings, but is otherwise similar.

Distribution: Brock's Creek, N.A., 22 Apr., 1929, T. G. Campbell, 1 ♂, 1 ♀, the latter labelled "caught on fresh horse manure"; Cairns, N.Q., A. P. Dodd, 1 ♀, labelled "ex corn"; Townsville, N.Q., F. H. Taylor, 2 ♀♀; Broadwater, N.S.W., Sept., 1928, D. S. North, 1 ♀; Nyngan, N.S.W., J. W. T. Armstrong, 1 ♂ (coll. Ferguson); Sydney, N.S.W., 8 Jan., 1923, Ferguson, 1 ♀ (coll. Ferguson); Wahroonga, near Sydney, N.S.W., 20 Nov., 1926, Ferguson, 1 ♀ (coll. Ferguson).

GRAPHOMYIA CAMPBELLI, n. sp.

A small, distinctively marked species, which appears to be nearest to *G. fascigera* Stein from Sumatra (*Tijdschr. Entom.*, lxii, Suppl., 1920, 66), from which it is, however, abundantly distinguished by the absence of any dark spot on the squame, by the presence of two posterior sterno-pleural bristles, by the entirely dark legs and by the abdominal markings.

♂. Eyes separated by about one-twelfth of the head-width, densely covered with pale brown hairs, which are longer than those of *G. maculata rufitibia* Stein. Frons black, linear above and widening to a narrow triangle above the antennae; frontal bristles eight, ending about three-fifths of distance from lower end of frons, interspersed with fine black hairs which extend the full length of frons. Parafrontals and parafacials white. Antennae brownish-black, third segment slightly paler; arista dark brown. Face dark brown, covered with ashy tomentum; cheeks with a brown patch near anterior margin, remainder ashy with a yellowish patch below the brown patch; two strong bristles at vibrissal angle and another just above; cheeks with numerous strong black hairs. Proboscis and palpi dark brown to black. Occiput black, with a narrow white postocular stripe which widens laterally.

Thorax covered with silvery tomentum, which is so reduced by the black markings as to form narrow dorsocentral and lateral stripes. The median and submedian black stripes of *G. maculata* Scop. are so broadened in this species as to have become almost completely confluent, forming a very broad median stripe, which narrows abruptly to half its anterior width midway between the suture and the scutellum; in certain lights the median area in front of the suture can be seen to be composed of a very narrow black median stripe bordered by grey. The sublateral black stripes are broad and continuous and are not interrupted at the suture. Scutellum covered with silvery tomentum, with a broad triangular black patch covering three-quarters of its extent, and with black side margins. Dorsocentrals 2+4. Pleurae grey, with brown to black patches; bristles and hairs black.

Abdomen orange, with black markings and patches of pale yellow tomentum. First visible tergite blackish-brown, with a faint greyish patch on each side of mid line towards apex. Second tergite with a narrow median black stripe, and with narrow submedian and broader sublateral pale yellowish patches overlying the orange. Third tergite similar to the second, but with, in addition, a pair of apical submedian black spots. Fourth tergite mainly covered with pale yellowish tomentum. Ventral aspects of tergites pale orange and silvery; sternites brown.

Wings hyaline, veins brown; hairs on vein R_{1+5} restricted to base below, extending one-quarter of distance to r–m above. Squames greyish-white, with creamy borders. Halteres pale yellow. Legs black, femora with some ashy tomentum. Length, 6 mm.

Holotype ♂. Brock's Creek, North Australia, 21 Apr., 1929, T. G. Campbell, in the collection of the Division of Economic Entomology, Canberra.

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE ACTINOMYCETALES. IV.

The Identity of Certain Species of Mycobacterium and Proactinomyces.

By H. L. Jensen, Macleay Bacteriologist to the Society.

(Four Text-figures.)

[Read 30th November, 1932.]

In a previous paper (Jensen, 1931) the present writer showed that two microorganisms previously classified as *Mycobacterium actinomorphum* and *Mycobacterium agreste* should correctly be placed in the new genus *Proactinomyces*. Since then, some work has been carried out on the morphology and biology of saprophytic mycobacteria and corynebacteria isolated from Australian soils, and authentic cultures of a number of related organisms have been obtained for comparison. Several of these have, on closer examination, been found, like the two organisms mentioned above, to belong to *Proactinomyces*, as previously defined (Jensen, 1931): organisms starting their life-cycle with the formation of a definite, more or less extensive vegetative mycelium which sooner or later divides, by formation of septa, into more or less bacterium-like, rod-shaped to coccoid elements, and generally producing an aerial mycelium in which no definite spores are formed. The following strains were examined: 1. *Mycobacterium agreste* Gray and Thornton (1928); 2. *M. crystallophagum* Gray and Thornton (1928); 3. *M. erythropolis* Gray and Thornton (1928); and 4. *Bacillus mycoides corallinus* Hefferan (1904),* from the National Collection of Type Cultures, Lister Institute of Preventive Medicine, London; 5. *Mycobacterium salmonicolor* den Dooren de Jong (1927); and 6. *M. opacum* den Dooren de Jong (1927), from the laboratory of Keuringsdienst van Waren, Rotterdam, Holland; and 7. *Microbacterium mesentericum* Orla-Jensen (1919), from the Biotechnical-Chemical Laboratory, Polytechnical School, Copenhagen, Denmark.

The media and the methods for cultivation and study were the same as described in the previous paper (Jensen, 1931), except that nutrient agar with 1% soluble starch was used for testing the diastatic activity.

Descriptions of the Organisms.

Proactinomyces corallinus (Hefferan), n. comb.

Synonyms: *Bacillus mycoides corallinus* Hefferan (1904).—*Serratia corallina* (Hefferan) Bergey (1923–30).—*Streptothrix corallinus* (Hefferan) Reader (1926).—*Mycobacterium agreste* Gray and Thornton (1928).—*Actinomyces agreste* (Gr. and Th.) Bergey (1930).—*Proactinomyces agrestis* (Gr. and Th.) Jensen (1931).

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* This organism was identified as a "*Streptothrix*" by Reader (1926).

Six strains were compared:

1. *Bac. corallinus* Hefferan } from the Lister Institute.
2. *Myc. agreste* Gr. and Th. }
3. AII, from garden soil, Sydney University.
4. Sc, from humus soil from Scone, N.S.W.
5. 271 } from red loam soils from the Riverina district, N.S.W.
6. 276 }

Since this comparison, as shown below, proved all the strains to be identical in all essential points, they must apparently be regarded as one single species, for which *corallinus*, on the grounds of priority, must be accepted as the valid specific name.*

Morphology.—All strains, like those previously described (Jensen, 1931), show by direct agar-microscopy according to the method of Ørskov (1923) essentially the same mode of development on dextrose-asparagine-agar: after 1–2 days small branching mycelia which after 2–3 days divide into rods of various length and arranged in angular positions, and later into quite coccoid elements. This process of division starts in the interior of the colonies and proceeds gradually towards the edges; the young colonies have a characteristic star- or burr-like appearance owing to a number of rhizoid projections which are the last to undergo division. Young mycelia show constantly a number of small refractive external granules which by high focussing are seen to rise into the air, and which doubtless represent a rudimentary aerial mycelium; they disappear again within a few days. Text-figure 1 shows the main features of the cycle of development. The rapidity with which this cycle is passed through varies considerably with the medium and the temperature (cf. Reader, 1926), and to some extent also with the strain. For instance, strains Sc and 271 appear almost exclusively as cocci on dextrose-nutrient-agar after 24 hours at 28–30° C., whereas strain 276 shows less tendency to coccus-formation, but a pronounced belt-staining. The cells of the freshly isolated strains were 1·0–1·2μ thick, as also reported by Gray and Thornton (1928); in *Myc. agreste* and *Bac. corallinus* they were somewhat thinner, probably owing to the longer period of artificial cultivation (cf. Hefferan (1904), who reports that the cells of the freshly isolated "*Bac. mycoides corallinus*" were originally as big as those of the anthrax bacillus, but that they gradually decreased somewhat in size). In the latter strain they were also somewhat more curved than in the others. None of the strains was acid-fast in nutrient or synthetic agar (as also found by Gray and Thornton), but in milk they exhibited a partial acid-fastness after 3–7 days.

Cultural characters.—The type of growth in various media is shown in Table 1. There are no very appreciable cultural differences between the strains. The intensity of the pigment varies somewhat, as was also the case with the 74 strains studied by Gray and Thornton (1928). The quantitative differences in the vigour of growth and the formation of a soluble yellow pigment in one strain (271) would hardly justify a separation into different species.

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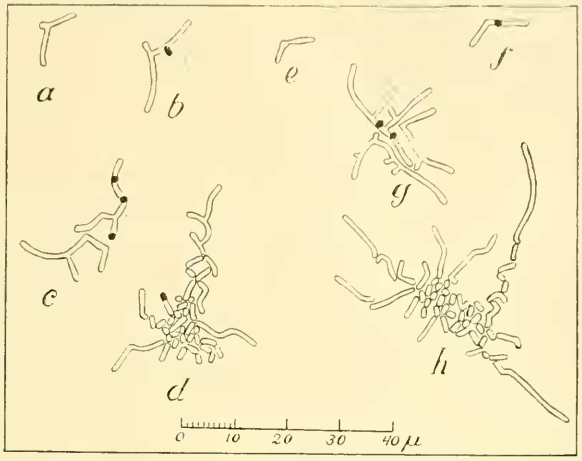
* I cannot here forgo the remark that Bergey's (1923-30) morphological description of *Serratia corallina* (syn. *Bac. mycoides corallinus* Hefferan) disagrees entirely with the descriptions by Hefferan (1904) and Reader (1926). Bergey characterizes it as a small, motile, gram-negative rod with one polar flagellum. One cannot help wondering whether the description has not been confused with that of *Bac. corallinus* Slater (1891), a non-spore-forming, motile, red-pigmented organism which would seem entitled to the name *Serratia corallina* according to Bergey's system of classification.

TABLE 1.—*Comparative Cultural Features of Strains of* Proactinomyces corallinus.

Medium.	*Mycobacterium agreste.*	*Bacillus mycoides corallinus.*	AII.	276.	Sc.	271.
Dextrose-asparagin-agar. 28–30° C.	Good, restricted, convex, myceloid edges, folded, pinkish-orange, becoming pale coral-red.	Good, restricted, convex, myceloid edges, slightly folded, pale pinkish-orange.	Abundant, convex, smooth, glistening, cream-coloured becoming pale pink.	Abundant, convex, smooth, glistening, pale pink, later greyish-orange.	Fair, restricted, myceloid edges, smooth, glistening, pale pink.	Fair, restricted, convex, becoming folded, pink, with yellow soluble pigment.
Dextrose nutrient agar. 28–30° C.	Good, restricted, convex, folded, undulate edges, pinkish cream-coloured, later deep coral-red.	Good, restricted, convex, folded, undulate edges, cream-coloured, later pale coral-red.	Similar to dextrose-asparagin-agar, still more abundant, pinkish-orange.	Similar to dextrose-asparagin-agar, still more abundant, grey pinkish-orange.	Good, convex, myceloid edges, smooth, soft, pink, later becoming coral-red.	Good, convex, myceloid edges, soft, smooth, pink, becoming deep red.
Potato. 28–30° C.	Good, spreading, granular, pink, later deep orange.	Good, spreading, raised, granular, orange.	Fair, spreading, raised, granular, dull greyish-orange.	Good, spreading, raised, granular, glistening, greyish-orange.	Good, spreading, smooth, glistening, greyish-pink.	Fair, spreading, granular, greyish-pink with yellow tinge.
Broth. 28–30° C.	Turbid, with voluminous pinkish-cream coloured sediment and fragile surface scum.	Slightly turbid with pinkish-cream-coloured surface granules and voluminous sediment.	Turbid, later becoming clear, with thick fragile cream-coloured scum and sediment.	Turbid with voluminous pinkish-cream-coloured sediment; no surface growth.	Clear with pink sediment and surface granules, later forming a pellicle.	Turbid, later becoming clear, with cream-coloured sediment, becoming pink.
Milk. 28–30° C.	Pink flakes and granules, forming a red sediment; milk slightly cleared in old cultures (6–8 weeks).	Pink granules and sediment, becoming red; milk slightly cleared in old cultures, yellowish.	Greyish-yellow to pinkish flakes and sediment; milk semi-transparent in old cultures.	Greyish-yellow to pink flakes and sediment; milk semi-transparent in old cultures.	Pink pellicle, later red sediment; milk viscid and semi-transparent in old cultures.	Pink sediment and granules; milk very slightly cleared in old cultures.
	(Clearing of milk not due to proteolytic action; formol-titration shows no increase in amino-N.)					
Nutrient gelatin. 16–18° C.	Both strains identical; filiform, granular, yellowish growth in stab; raised, wrinkled, red surface colony; no liquefaction.		No liquefaction.	No liquefaction.	No liquefaction.	No liquefaction.



Text-fig. 1.—Development of *Proact. corallinus* on dextrose-asparagine-agar at 16-18° C. *a*, *Bac. mycoides corallinus*, 18 h.; *b*, same, 23 h.; *c*, same, 42 h.; *d*, same, 3 days; *e*, *Myc. agreste*, 18 h.; *f*, same, 23 h.; *g*, same, 42 h.; *h*, same, 3 days. × 700. Aerial mycelium heavily shaded.

Physiological features are shown in Table 2. There is a certain amount of variation here, but not more than among the 74 strains studied by Gray and Thornton, which were obtained by a selective method (accumulation in a nutrient solution with phenol or cresol as the sole source of carbon) and therefore all capable of decomposing aromatic compounds. It might perhaps be

TABLE 2.—*Comparative Physiological Features of Strains of* Proactinomyces corallinus.

	Bacillus corallinus.	*Mycobacterium agreste.*	AII.	276.	Sc.	271.
Proteolytic action ..	−	−	−	−	−	−
Diastatic action	−	−	−	−		?
Invertase action	−	−	−	−	−	−
Decomposition of cellulose	−	−	−	−	−	−
Decomposition of phenol	−	+	?	−	−	?
Utilization of paraffin ..	+	+	+	+	+	+
Utilization of N as*:						
$NaNO_3$	2	2	3	3	2	3
$(NH_4)_2HPO_4$ ..	2	3	3	4	2	4
Asparagine ..	3	3	4	4	2	4
Peptone	3	4	4	5	4	4
Reduction of nitrate ..	+	+	−	?	+	+
Formation of indol† ..	+	+	−	+	+	+
Acid in dextrose-broth ..	−	−	−	−	−	−
Acid in glycerin-broth ..	−	−	−	−	−	−
Growth anaerobically ..	−	−	−	−	−	−

*Basic solution: Dextrose 1·0%; K_2HPO_4 0·1%; $MgSO_4$ 0·05%; NaCl 0·05%; in distilled water; N-compound 0·2%. Character for growth: 0, no growth; 1, trace or very scant; 2, scant; 3, fair; 4, good; 5, excellent.

†By Salkowski's test.

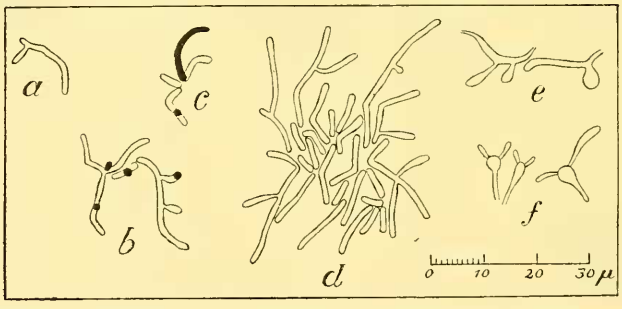
suggested to retain the name *agrestis* for the phenol-decomposing strains, but it would hardly be logical to give this property a special preference before other physiological properties (cf. Gray and Thornton, 1928).

PROACTINOMYCES SALMONICOLOR (den Dooren de Jong), n. comb.

Synonyms: *Mycobacterium salmonicolor* den Dooren de Jong (1927).—*Flavobacterium salmonicolor* (den Dooren de Jong) Bergey (1930).

Morphology.—This organism, of which den Dooren de Jong gives a rather incomplete description, is closely related to *Proact. corallinus*. On dextrose-asparagine-agar after 18–24 hours at 18–20° C., long branching rods are formed, 1·0–1·3μ thick, with small refractive granules of aerial mycelium, sometimes stretching into quite long filaments; after 2–3 days small definite mycelia are present, and after 5–6 days these have largely divided into short rods and cocci; the colonies have the same burr-like appearance as those of *Proact. corallinus*. Many cells at the edge of the colonies show, after 3–4 days, club- or pear-shaped swellings, up to 2·5–3·0μ thick; after 5–6 days many of these swollen cells are seen to "germinate" with the formation of two more slender sprouts (Ørskov (1923) gives an almost identical-looking picture of "*Streptothrix rubra*"; it is questionable, indeed, whether these two organisms are not really identical). At 28–30° C. the development is more rapid. In dextrose-asparagine solution we find, after 20 hours, long branched rods, 1·0–1·4μ thick and up to 30–35μ long, but after 2 days irregular, club- or pear-shaped rods looking like big diphtheroids. In dextrose-nutrient-agar only short rods and cocci, 1·2–1·5μ, are found after 2 days at 30° C., but in milk the long branching rods are still present after 3 days. The organism is not acid-fast in synthetic media or in young cultures on nutrient agar, but partly so in milk after 3–7 days and nutrient agar after 4–6 weeks.

The course of development is shown in Text-figure 2.



Text-fig. 2.—Development of *Proact. salmonicolor* on dextrose-asparagine-agar at 16-18° C. *a*, 18 h.; *b*, 23 h.; *c*, same, long filament of aerial mycelium; *d*, 44 h.; *e*, 3 days; *f*, 7 days. × 700. Aerial mycelium heavily shaded.

Cultural characters.—Cultivation at 28–30° C., unless otherwise stated. *Dextrose-asparagine-agar*: Good growth, restricted, rather flat, edges lobate, surface warty, glistening, first pale orange, later pure ochre-yellow; consistence crumbly. After 5–6 weeks the growth is paler with many small round raised yellow "secondary colonies"; cultures obtained by plating from these do not seem to differ from the original. *Dextrose-nutrient-agar*: Excellent growth, spreading, flat, dense, edges lobate, surface folded, glistening, yellow gradually

changing to deep orange-red. *Potato*: Good growth, raised, warty, crumbly, glistening, at first buff, changing to orange and finally to almost blood-red. *Nutrient gelatin*, 20–22° C.: Scant arborescent growth in stab; small wrinkled orange surface colony; no liquefaction. *Nutrient broth*: Fair growth; thin pellicle and granular sediment, at first cream-coloured, later red; broth clear at first, slightly turbid after 3 weeks. *Milk*: Good growth; pellicle of small cream-coloured granules after 2 days, later a thick orange sediment; milk is not coagulated, but appears slightly cleared after 5 weeks, the reaction becoming alkaline.

Physiological features.—Saccharose is not inverted, although readily utilized with sodium nitrate as a source of nitrogen. Starch is not hydrolyzed. Cellulose is not decomposed. Paraffin is readily utilized as a source of carbon. Phenol is not utilized. Nitrate is reduced to nitrite. Indol is not formed. No acid is formed from dextrose or glycerin. No growth in oxygen-free atmosphere. Nitrate, ammonium salts, asparagine and peptone are utilized almost equally well with dextrose as source of carbon, although the growth is most rapid with peptone.

The morphology of this organism shows conclusively that Bergey's (1930) classification of it as *Flavobacterium* is unjustified. This genus comprises small non-spore-forming, usually gram-negative rods, characterized by formation of a yellow pigment and by feeble powers of attacking carbohydrates, gas never being formed and acids rarely. Bergey omits to mention the tendency to branching, which den Dooren de Jong (1927) states to be present, and moreover the present organism produces a luxuriant growth on dextrose and saccharose with inorganic sources of nitrogen; this can hardly be called a "feeble power of attacking carbohydrates", since it is not fair to gauge this power by the formation of acids or gas in the case of organisms which oxidize carbohydrates completely to carbon dioxide and water (cf. Merrill, 1930).

Proactinomyces opacus (den Dooren de Jong), n. comb.

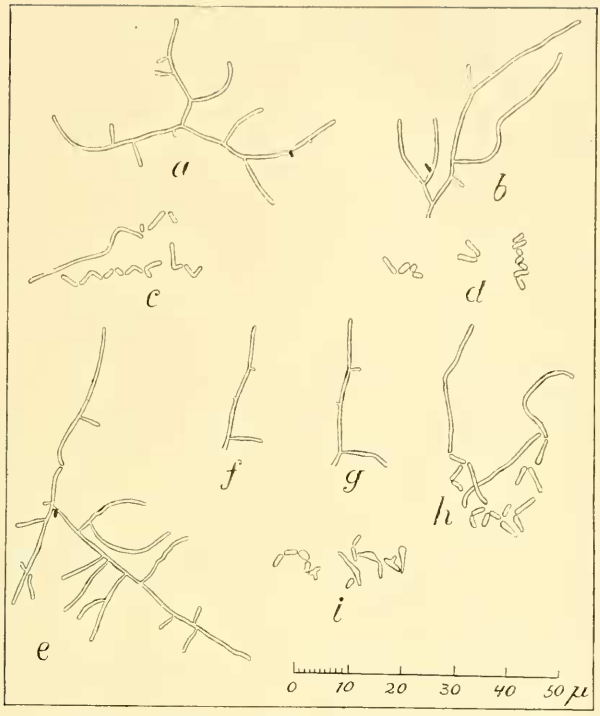
Synonyms: *Mycobacterium opacum* den Dooren de Jong (1927).—*Mycobacterium crystallophagum* Gray and Thornton (1928).—*Actinomyces crystallophagus* (Gr. and Th.) Bergey (1930).

Morphology.—The two strains *Myc. opacum* and *Myc. crystallophagum* are morphologically identical. They form in dextrose-asparagine-agar after 1–2 days at 16–18° C., quite extensive mycelia composed of richly branching hyphae, 0·6–0·9μ thick; short, simple filaments of aerial mycelium are seen, but no spores are formed in these. The mycelia are fragile and appear in stained preparations mostly as branched filaments of varying length. After 3–4 days the young colonies are round, dome-shaped, and surrounded by a flat fringe of long branching filaments which, during the following days, undergo a division giving rise to rod-shaped cells in angular arrangement and gradually growing shorter, finally quite coccoid. Text-figure 3 shows the course of development. At 28–30° C. the development is similar, but more rapid; this is also the case in nutrient agar, milk and potato. There is at the higher temperatures a tendency to formation of swollen, club-like cells, and the strain *opacum* is somewhat less prone to the formation of typical cocci than the other. Both strains exhibit a partial acid-fastness in milk, *opacum* also to a slight extent in nutrient agar.

TABLE 3.—*Comparative Cultural Features of Strains of* Proactinomyces opacus *and* Proactinomyces erythropolis.

Medium.	*Mycobacterium opacum.*	*Mycobacterium crystallophagum.*	*Mycobacterium erythropolis.*
Dextrose-asparagine-agar. 28–30° C.	Good growth, raised, spreading, edges myceloid, surface folded, pale cream-coloured changing to pale pink; consistence firm, pasty.	Abundant growth, spreading, raised, edges myceloid, surface folded, glistening, white with pale pink tinge; consistence soft, pasty.	Good growth, raised, spreading, edges lobate, surface highly folded, dull, white, becoming pinkish-cream-coloured; consistence firm, pasty.
Dextrose nutrient agar. 28–30° C.	Good growth, raised, spreading, surface folded and granulated, dull white changing to pale buff; consistence rather dry and coherent.	Abundant growth, restricted, raised, edges lobate, surface highly folded, white, glistening, changing to pale buff; consistence pasty.	Abundant growth, spreading, convex, edges entire, surface smooth, glistening, pinkish-cream-coloured; consistence soft and moist.
Potato. 28–30° C.	Good growth, raised, restricted, surface highly folded (lichnoid), dull cream-coloured, becoming greyish-pink; consistence curd-like.	Good growth, raised, spreading, surface highly folded and wrinkled, pale cream-coloured, later pink tinge; consistence curd-like.	Good growth, convex, spreading, surface slightly folded, glistening, pinkish-cream-coloured; consistence soft and moist.
Nutrient gelatin. 16–18° C.	Scant growth in stab, white, filiform, later finely arborescent; small white wrinkled surface colony; no liquefaction.	Scant growth in stab, cream-coloured, finely granulated; small raised and wrinkled pinkish-white surface colony; no liquefaction.	Scant growth in stab, cream-coloured, filiform; small raised and finely wrinkled pinkish-white surface colony; no liquefaction.
Nutrient broth. 28–30° C.	Thin, silky, white pellicle developing into a thick fragile cream-coloured scum; voluminous sediment of same colour; broth remains clear.	Thin white pellicle developing into a thick fragile cream-coloured scum; voluminous sediment of same colour; broth at first turbid, later clear.	Flaky pinkish-white sediment and surface scum, becoming cream-coloured; broth turbid, becoming clear after two weeks.
Milk. 28–30° C.	White, later pale pink to cream-coloured flakes and granules, forming a voluminous sediment; milk partially cleared in old cultures; reaction alkaline.	White to cream-coloured flakes and granules along the tube, voluminous cream-coloured sediment; milk very slowly cleared, becoming viscid in old cultures.	Pinkish-cream-coloured flakes and granules along the tube; voluminous sediment of the same colour; milk slowly and gradually cleared, becoming viscid in old cultures.

(The characteristic changes brought about in milk cultures by these strains as well as other non-proteolytic proactinomycetes do not indicate a digestion due to proteolytic action, since formol-titration does not show any increase in the content of amino-N.)



Text-fig. 3.—Development of *Proact. opacus* on dextrose-asparagine-agar at 16-18° C. *a, Myc. opacum,* 24 h.; *b,* 44 h., edge of colony; *c,* 6 days, edge of colony; *d,* 7 days, 28° C.; *e, Myc. crystallophagum,* 44 h.; *f,* same specimen, 48 h.; *g,* same specimen, 50 h.; *h,* 6 days, edge of colony; *i,* 4 days, 28° C. × 700. Aerial mycelium heavily shaded.

Cultural characters.—As Table 3 shows, these two strains are also very similar in cultural respect; *crystallophagum* is somewhat more soft and moist than *opacum* and has more tendency to produce a turbidity in liquid media, where the latter grows mostly as large discrete flakes, leaving the medium clear. Possibly the two strains represent "plane" and "perrugose" varieties of the same species.

Physiological features, listed in Table 4, show a complete identity, except for the ability of *crystallophagum* to decompose phenol. However, here as well as in the case of *Proact. corallinus,* we may doubt whether this single physiological difference is sufficient for a species differentiation. It seems, therefore, that the two strains must be regarded as a single species, the valid name of which will be *Proactinomyces opacus.*

PROACTINOMYCES ERYTHROPOLIS (Gray and Thornton), n. comb.

Synonyms: *Mycobacterium erythropolis* Gray and Thornton (1928).—*Actinomyces erythropolis* (Gr. and Th.) Bergey (1930).

Morphology.—When grown on dextrose-asparagine-agar this organism is hardly distinguishable from the previous group, apart from a somewhat more pronounced tendency to production of swollen, club-shaped cells; in certain other media its

TABLE 4.—*Comparative Physiological Features of Strains of* Proactinomyces opacus *and* Proactinomyces erythropolis.

	Mycobacterium opacum.	*Mycobacterium crystallophagum.*	*Mycobacterium erythropolis.*
Proteolytic action	−	−	−
Diastatic action	−	−	−
Invertase action	−	−	−
Decomposition of cellulose	−	−	−
Decomposition of phenol	−	+	+
Utilization of paraffin	+	+	+
Utilization of N as*:			
$NaNO_3$	4	4	3
$(NH_4)_2HPO_4$	4	4	3
Asparagine	4	4	3
Peptone	5	4	4
Reduction of nitrate	+	+	−
Formation of indol	−	−	−
Acid in dextrose-broth	−	−	−
Acid in glycerin-broth	−	−	−
Growth anaerobically	−	−	−

* Basic solution: Dextrose 1·0%; K_2HPO_4 0·1%; $MgSO_4$ 0·05%; NaCl 0·05%; in distilled water; N-compound 0·2%. Character for growth: 0, no growth; 1, trace or very scant; 2, scant; 3, fair; 4, good; 5, excellent.

mycelial growth is more marked than is the case with the previous: in milk, long branching mycelia are present after 3–7 days, and no cocci are formed in broth (cf. Gray and Thornton). It is not acid-fast in synthetic or nutrient agar, but somewhat acid-fast in milk after 3 days.

Cultural and physiological features (see Tables 3 and 4) are similar to those of *Proact. opacus*, apart from the absence of nitrate reduction and a characteristic semi-transparent, watery growth on sugar-free nutrient agar (cf. Gray and Thornton, 1928).

The last three strains (*opacum*, *crystallophagum*, and *erythropolis*) show by direct microscopical examination of the young agar colonies a very clear picture of the formation of mycelial branches (cf. Jensen, 1931): minute granules appear outside the hyphae, grow into a small pear-shaped bud attached to the main stem by a thin stalk, and stretch into a lateral branch (see Text-figure 3, *e–g*).* One cannot help being struck by the resemblance of this phenomenon to the formation of what are described as "reproductive bodies" by Löhnis (1921); the pictures of *Myc. tuberculosis* according to Meirowsky and of *Bact. coli* according to Hort, as reproduced by Löhnis, are particularly instructive, as well as the more recent observations by Cunningham (1931) on "reproductive bodies" in *Bac. saccharobutyricus* and by Stoughton (1929) on "stalked gonidia" in *Bact. malvacearum.* Löhnis (1921) describes the phenomenon in the following words:

"If the gonidia are not liberated by the partial or complete dissolution of the cell wall, but remain confined within the cell, they develop into either buds or branches" (p. 127). . . . "Two to four or more gonidia may be produced within

* Reader (1926) gives a microphotograph showing exactly the same phenomenon in a young culture of *Proact. corallinus.*

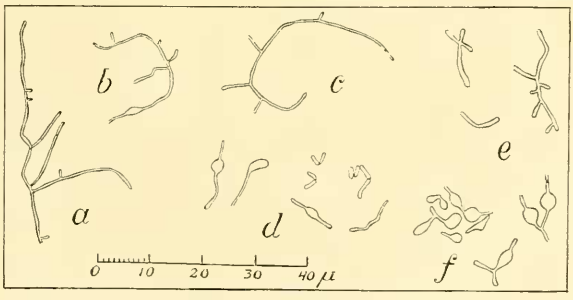
each cell; . . . They may start growing while still within the parent cell, forming buds and branches or directly new vegetative cells within the cell membrane. . . Sometimes they remain temporarily attached to the parent cell by a comparatively long stem" (p. 163).

While the final judgment on the nature of these phenomena may still be held in suspense, there can be no doubt as to their objective existence, and the conclusion might not be unjustified, that this formation of alleged reproductive bodies and gonidia in the "true" bacteria is a phenomenon homologous to the formation of branches in *Proactinomyces* and *Actinomyces*, and the spore formation in *Micromonospora*.

PROACTINOMYCES MESENTERICUS (Orla-Jensen), n. comb.

Synonym: *Microbacterium mesentericum* Orla-Jensen (1919).

Morphology.—This organism, of which Orla-Jensen gives a morphologically and culturally rather incomplete description, proved to be a typical *Proactinomyces*. On dextrose-asparagine-agar, nutrient agar, Saboureaud's agar* and broth it grows at 16–18° C. as extensive mycelia composed of richly branching hyphae of a somewhat variable thickness, 0·4–0·8μ; no aerial hyphae are seen. With increasing age the hyphae divide into fragments of varying size and shape, partly diphtheroid rods, but no real cocci. There is, particularly in the richer media, a tendency to formation of large, swollen, fusiform to almost spherical cells, up to 3·5μ in diameter and staining intensely with carbol fuchsin; when transferred to fresh media, they germinate and produce a new mycelium. On nutrient agar at 28–30° C. the organism appears after 1 day exclusively as irregular, branching rods of varying thickness (cf. Orla-Jensen, 1919, Pl. L). After 2 days and in older cultures the microscopical picture is entirely dominated by big lemon-shaped to spherical swollen cells. In milk, long rods and even definite mycelia are still seen at 30° C. after 10 days. The various cell types are reproduced in Text-figure 4. There is no acid-fastness in any medium. It is characteristic of this as well as of other non-acid-fast proactinomycetes (*Proact. actinomorphus* and *flavescens*, Jensen, 1931), that their cells, when examined by the direct agar-microscopy method of Ørskov (1923), are much less refractive to the light than those of previously described partially acid-fast organisms.



Text-fig. 4.—*Proact. mesentericus.* *a*, dextrose-asparagine-agar, 20 h. 16° C.; *b*, same, 4 days 16° C.; *c*, Saboureaud's agar, 24 h. 16° C.; *d*, same, 4 days 16° C.; *e*, dextrose-nutrient-agar, 20 h. 28° C.; *f*, same, 3 days 28° C. × 700.

* Milk is boiled for 5 min. with 0·2% HCl, and filtrated; the filtrate is neutralized, and added 1% peptone, 1% dextrose, 0·3% urea, and 1·5% agar.

Cultural characters.—This organism grows decidedly better at 16–18 than at 28–30° C.; the following description, therefore, refers to the former temperature unless otherwise stated.—*Dextrose-asparagine-agar*: Fair growth, narrow, raised, granular, very pale yellow, glistening; condensation water clear, with small granules. At 30° C. only scant growth consisting of small irregular white granules, growing deeply down into the agar.—*Dextrose-nutrient-agar*: Good growth, restricted, with undulate edges, surface with high transversal folds, cream-coloured; the consistence is firm and cartilaginous after 2 days, later more loose and brittle. Growth at 28–30° C. rather scant; smooth, soft, glistening, cream-coloured smear.—*Saboureaud's agar*: Excellent growth, spreading, at first flat and smooth, pale straw-yellow, perfectly hard and cartilaginous, later raised and strongly folded, of a loose, curd-like consistence, bright lemon-yellow. Growth at 28–30° C. only fair, restricted, folded, cream-coloured, soon becoming soft and smeary.—*Potato*: Scant growth; restricted, soft, cream-coloured smear.—*Nutrient gelatin*: Good growth; finely arborescent, cream-coloured growth in the stab; raised, folded, pale yellow surface colony. No liquefaction.—*Broth*: Good growth; voluminous, flaky, whitish sediment; broth clear.—*Milk*: 28–30° C. Small cream-coloured granules along the tube; the milk undergoes no visible changes within 4 weeks. No proteolytic action.

Physiological features.—Saccharose is inverted. Starch is hydrolyzed. Cellulose is not decomposed. Nitrate is reduced to nitrite. Indol is not formed. No growth in oxygen-free atmosphere. N is utilized as sodium nitrate, ammonium phosphate, and asparagine, although these are inferior to peptone as sources of N. The fermentative properties of this organism were studied in detail by Orla-Jensen.

Key to Identification of Species of Proactinomyces.

The species of *Proactinomyces* described here and in a previous paper (Jensen, 1931) may be classified according to the following key:

I. Partially acid-fast organisms with strongly refractive cells. Non-proteolytic and generally non-diastatic; constantly capable of utilizing paraffin.
  A. Initial mycelia very small, rapidly dividing into rods and cocci. (Transition to *Mycobacterium.*)
    1. Slowly growing organism; cells 0·5-0·7μ in diameter ..... *Proactinomyces minimus*
    2. Rapidly growing organisms; cells 1·0-1·2μ in diameter.
      a. Cystites* not formed. Rapid formation of cocci ..... *Proactinomyces corallinus*
      b. Cystites formed. Less rapid formation of cocci ..... *Proactinomyces salmonicolor*
  B. Initial mycelia well developed, richly branching, dividing into rods and generally into cocci.
    1. Vegetative mycelium soft, without macroscopically visible aerial mycelium.
      a. Vegetative mycelium red; may produce variants with undivided vegetative mycelium and visible white aerial mycelium, or yellow and white variants ..... *Proactinomyces polychromogenes*
      b. Vegetative mycelium white to pale pink.
        x. Growth on nutrient agar opaque, cream-coloured; cocci in broth culture ..... *Proactinomyces opacus*
        xx. Growth on sugar-free nutrient agar watery; no cocci in broth culture ..... *Proactinomyces erythropolis*

* In the present writer's opinion, the term "cystites" (Enderlein, 1925) may conveniently be used as a collective term for the swollen cells which characterize many of these organisms as well as the corynebacteria, without it being necessary to commit oneself to Enderlein's definition of them as cells with a "polydynamic elemental nucleus" (polydynamen Mych.).

2. Vegetative mycelium hard, yellow, with white aerial mycelium; hyphae divide into chains of acid-fast cocci ..... *Proactinomyces paraffinae*

II. Non-acid-fast organisms with weakly refractive cells; no distinct formation of cocci. Constantly diastatic.

A. Non-proteolytic. No aerial mycelium; marked formation of cystites .... *Proactinomyces mesentericus*

B. Proteolytic organisms.

1. Growth on nutrient agar with rapid formation of unbranched diphtheroid-like rods; no typical cystites; broth turbid .... *Proactinomyces actinomorphus*

2. Growth on nutrient agar with extensive mycelia; simple unbranched rods not formed; cystites present. Broth clear .... *Proactinomyces flavescens* (*Transition to Actinomyces.*)

The pathogenic *Act.* (*Proact.*) *asteroides*, *caprae*, and *farcinicus* obviously belong to Group IB. The same is doubtless the case with numerous other acid-fast, non-proteolytic actinomycetes isolated from and possibly etiologically connected with actinomycotic affections. Such organisms have been described by Cornwall and Lafrenais (1922), Pijper and Pullinger (1927), Kulikowska (1930), and numerous earlier authors summarized by Henrici and Gardner (1921). The fact that similar organisms occur as widespread saprophytic forms suggests that they might easily be encountered as secondary infections in morbid affections. In Group IA we would probably have to place the organisms studied by Vierling (1921; cf. Haag (1927) who recognized as actinomycetes a number of paraffin-decomposing, weakly acid-fast, mycobacterium-like organisms similar to those studied by Vierling).

Summary.

A number of organisms previously described as species of *Mycobacterium* were found, on account of their definite mycelial growth in the initial stages of their life cycles, to have their proper place in the genus *Proactinomyces*.—*Myc. agreste* Gray and Thornton and *Bac. mycoides corallinus* Hefferan were found to be so similar that they must be regarded as one species, *Proact. corallinus*.—*Myc. salmonicolor* den Dooren de Jong is closely related to this and should be called *Proact. salmonicolor*.—*Myc. opacum* den Dooren de Jong and *Myc. crystallophagum* Gray and Thornton proved to be identical; this species should be called *Proact. opacus*.—*Myc. erythropolis* is closely related to this; its proper name should be *Proact. erythropolis*.—*Microbacterium mesentericum* Orla-Jensen showed a very distinct mycelial growth and should be called *Proact. mesentericus*.—These organisms, together with some species, previously described by the present writer, form two separate groups. Group I consists of non-proteolytic organisms with strongly refractive cells showing a partial acid-fastness in milk and sometimes in other media, and constantly capable of decomposing paraffin; some species of this group form a transition to *Mycobacterium*. Group II comprises mostly proteolytic forms with weakly refractive, non-acid-fast cells; from this group there is a close transition to *Actinomyces*.

The author wishes to express his thanks to Dr. R. St. John-Brooks, Curator, Lister Institute of Preventive Medicine, London, to Dr. L. E. den Dooren de Jong, Keuringsdienst van Waren, Rotterdam, Holland, and to Mr. C. Ledoux, Biotechnical-Chemical Laboratory, Polytechnical School, Copenhagen, Denmark, for their courtesy in forwarding cultures of the organisms examined here.

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ON THE GROWTH AND REACTION TO GRAZING OF THE PERENNIAL SALTBUSH, *ATRIPLEX VESICARIUM*. AN ECOLOGICAL STUDY OF THE BIOTIC FACTOR.

By T. G. B. Osborn, University of Sydney; J. G. Wood, University of Adelaide; and T. B. Paltridge, Field Officer, Council for Scientific and Industrial Research.

(Plates vii–ix; five Text-figures.)

[Read 30th November, 1932.]

In this paper, the second of a series[1] dealing with investigations at the Koonamore Vegetation Reserve, the dominant plant over much of the shrub steppe formation (*Atriplex vesicarium*, the perennial saltbush) is discussed in relation to its arid environment. In particular the results of a new biotic factor, grazing by sheep, are considered.

The location of the Koonamore Vegetation Reserve and the general purpose of the investigations have already been described (Osborn, 1925). It is the Arid Flora Research Station of the University of Adelaide, to whom it was given in 1925 by Messrs. Hamilton, Wilcox Ltd., the then owners of Koonamore.

In a paper to appear shortly we shall deal with the results of five years work on the Reserve. The present investigation has been conducted from this base and has been carried out at intervals during the period April, 1928, to June, 1931. During this time the work has been supported by a grant from the Commonwealth Council for Scientific and Industrial Research.[2] The investigations herein described could not have been carried out without the kindness of the owners of the neighbouring sheep stations who readily allowed us access to their paddocks. It is not possible to mention all to whom our thanks are due, but we are particularly indebted to R. E. H. Hope, Esq., owner of Koonamore, and his manager, Mr. J. Hardy; to the owners of Melton and their manager, Mr. W. Smith; to the directors of the Mutooroo Pastoral Company; and to C. Wade, Esq., of the Panarammattee Pastoral Company. To these, and the many others who have helped us, we offer our sincere thanks.

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[1] The first was "On the Autecology of *Stipa nitida*, a study of a fodder grass in Arid Australia". Proc. Linn. Soc. N.S.W., lvi, 1931, 299-324.

[2] My thanks are due to the Council for Scientific and Industrial Research for the generous grant which enabled the Koonamore investigations to be carried on upon an extended scale after my departure from Adelaide. I am also much indebted to the Council of the University of Adelaide for allowing me facilities for continued work at Koonamore after my appointment to Sydney.—T.G.B.O.

Introductory.

The harmful results that may follow from the grazing by stock upon the perennial flora in dry regions has been the subject of considerable study, particularly in the United States of America. As early as 1910 Griffiths showed, as a result of his observations on a protected stock range in Arizona, that the indigenous perennials, which were popularly supposed to be in process of being driven out by aggressive annuals, were really succumbing to overstocking. He found that the perennial vegetation would return with protection.

Sampson and Malmsten (1926) showed that frequent and close cropping by stock was detrimental to both perennials and herbaceous plants on Western forest reserves. It led to soil impoverishment and erosion. Their work showed, amongst other things, that rotational grazing was important in preserving the natural plant cover.

The methods involved in the study of the reaction of shrubby perennials to grazing must necessarily differ somewhat from those employed in the study of herbaceous flora. Nelson (1930), in a paper contributed to the symposium on Range Ecology held by the Ecological Society of America, described the quadrat methods used. We have utilized information gained from quadrats, of which we have established a number, some under complete protection, others subjected to grazing in the open paddocks. These will be described shortly, but for the purpose of the present inquiry we have found the method less suitable than a modification of the line transect described below. We have also utilized a metre quadrat frame dropped at regular intervals along transect lines, but this, too, has been found unsuitable for perennial shrubs of the *Atriplex* type. The community that we have investigated is too open and the plants too gregarious for a method of dropped quadrat sampling to give a reliable picture of the community. Such a method we have found valuable in the study of herbaceous plants, e.g., in our work on *Stipa* (Osborn, Wood and Paltridge, 1931).

Over considerable portions of arid Australia saltbush is the most valuable plant. It is true that when other forage is available (grass and "herbage" in "good seasons"), saltbush is taken by sheep in the way of a browse and is not consistently grazed. It is palatable because of its salt content, and its protein content is high. In time of drought, it is the only fodder plant available to sheep. Moreover, it is the only perennial ground cover over vast areas and its destruction leads to a calamitous amount of drift and erosion.

Since the settlement of Australia by white peoples, a profound change has occurred in the biota (Osborn, 1929). This is everywhere marked, but in the arid portions it is particularly severe. An indigenous flora, evolved in an environment in which close grazing animals were absent, has been subjected to more or less intense grazing by large stock, sheep in particular, and rabbits. The changes that may be produced by an unduly severe incidence of stocking are profound. These have frequently been referred to because in a severe state the cumulative influence of the stock effect and an arid climate may destroy the whole plant community. Hitherto no attempt has been made to evaluate just what changes do occur in the permanent flora as a result of this new biotic factor. The purpose of the present paper is to give an account of these changes.

The Saltbush Community.

The chief physiographic features of the north-east of South Australia are the rocky hills and the wide open valleys and peneplains between them. The

soils of the hills are shallow and show immature profiles. They are derived from the quartzites and mudstones of Upper and Lower Precambrian age which form the basis of the ranges. The vegetation consists of a climax desert scrub of various sclerophyllous and xeromorphic species dominated usually by *Acacia* spp.

The soils of the plains, or properly speaking plateaux, are derived from these hills under a modern cycle of arid erosion. As a result of internal drainage these soils contain a good deal of silt and may be classed as sandy loams. The profile is a mature one with much travertine limestone in the B horizon. An analysis and profile has been given by us in a previous communication (1931, p. 308).

On these soils and on the gentler slopes of the hills, *Atriplex vesicarium* forms a true shrub steppe, in the main forming a pure community (Pl. vii, fig. 1), but occasionally mixed with other species, especially of *Kochia*, some of which assume local dominance (Osborn and Wood, 1923). Trees are very rare, but occasional bushes of *Eremophila* spp. and *Cassia* spp. may be found when the water relations are suitable. Following rains, various grasses and a host of ephemeral plants, the "herbage" of the Australian pastoralist, are to be found. Many of these develop most abundantly in the mounds of sand accumulated around the base of the saltbush. However, the only true permanent vegetation is the saltbush itself, or its other chenopodiaceous allies.

Atriplex vesicarium is an erect shrub whose average height and diameter (the mean of some 5,000 observations) is 32 and 34 cm. respectively. Occasionally much larger bushes are found. One growing on quadrat 100 at the Koonamore Vegetation Reserve, under complete protection from grazing for 5 years, is 160 cm. in diameter, though only 30 cm. high. This is an unusually large and sprawling bush, but several others may be found on the quadrats in the Koonamore Vegetation Reserve that are more than 100 cm. in diameter and a few of them reach 50–55 cm. high.

The plant has the characteristic anomalous secondary thickening of the Chenopodiaceae. The stems are rarely as much as 1 cm. in diameter; they are usually much more slender twigs. At all ages the wood is brittle and under stress it snaps easily with an almost clean transverse fracture. It is, therefore, easily damaged by mechanical means.

Generally only very young plants show anything like a main axis. The seedling and young plant (Text-fig. 1) show the early development of many buds from the basal part of the stem. The majority of plants show a number of slender, freely branching stems rising from the base which is usually surrounded by a mound of fine soil and sand deposited by the wind. This is always the case on grazed country, but in virgin areas the light surface layer of soil is sometimes more evenly distributed. It will be readily understood that a low-growing free-branching shrub such as *Atriplex* will hold the soil and accumulate a mound about itself. However, the plant is not a hemicryptophyte, but is chamaephytic in its growth form.

The stems appear whitish-grey, even greenish-grey after rain, though many dead twigs are generally present. These are a dark grey when they lose their bark, which they do rapidly after death. The leaves are obovate to broad lanceolate, 1–2·5 cm. long and 0·5–1 cm. wide. They are rather thick and fleshy to the touch when turgid. The leaf is never really green, but always more or

less grey and scurfy-looking owing to its dense cover of non-cuticularized hairs. These give the leaf a mealy white appearance which becomes more pronounced as the leaf loses water. Wood (1923 and 1932*b*) has shown that the plant has a low transpiration rate and that the leaves resist desiccation owing to a pentosan colloid complex (1932). The wilted leaves are freely shed by the

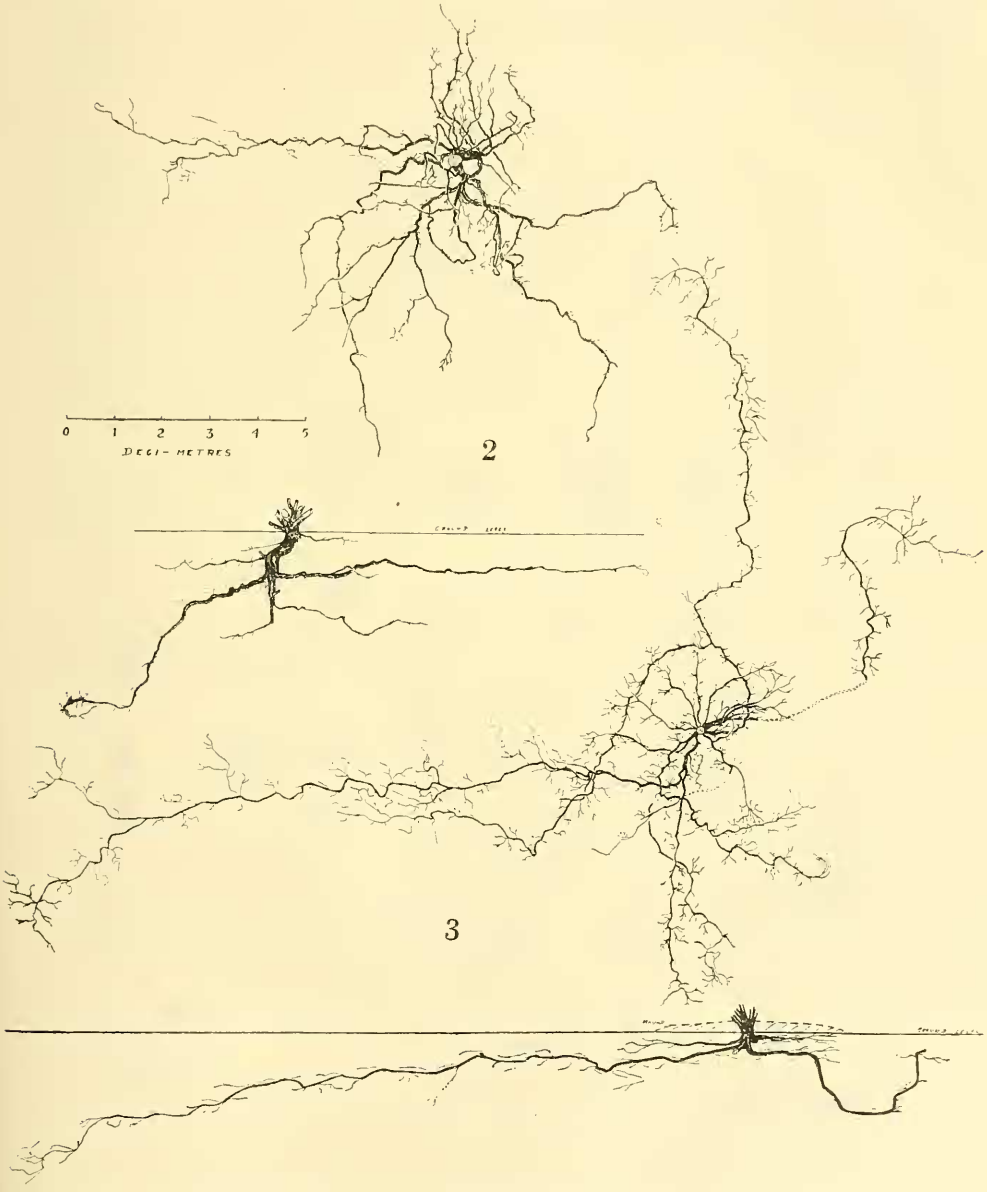
Text-fig. 1.—Seedling and young plant of *Atriplex vesicarium* showing early development of lateral shoots from the basal nodes. × ⅓. Miss C. Ure, *del.*

plants until a state of complete defoliation is reached (cf. Pl. vii, figs. 1 and 2, which shows an *Atriplex* covered peneplain in a wet season and during drought). At Frome Downs, a station about 60 miles to the north of Koonamore, which was visited by one of us (T.B.P.) in October, 1929, it was estimated, as a result of an extensive series of transect observations, that 95% of the plants were leafless.

These plants were in true virgin country, not yet stocked by sheep owing to lack of facilities for watering. Such plants are by no means all dead, like others described below; some would regenerate in good seasons throwing new shoots from the base of the stems. This drought deciduous habit is not usual in Australian arid plants, though it is paralleled in the case of such plants as *Astragalus* and *Fouquieria* (Maximov and Yapp, 1929).

Unlike that of most perennial plants in arid regions, the root system of *Atriplex vesicarium* is very shallow, never penetrating the nodular layer of travertine limestone, and mostly living within 10–20 cm. of the surface. It will be seen from Text-figures 2 and 3 that the surface extension is considerable, some of the longer roots being 2 metres in length. There is no tap-root, but a number of spreading laterals which branch at intervals. These tertiary members rarely branch, but produce numerous groups of short-lived feeding roots which are deciduous in time of drought, and renewed in wet periods.

The root system is probably largely non-functional during prolonged dry periods, but the plant possesses a secondary method of water absorption through its leaves. Wood (1925) has shown that shoots of *Atriplex vesicarium* gain weight when kept in an atmosphere of 85% saturation with water, a figure that

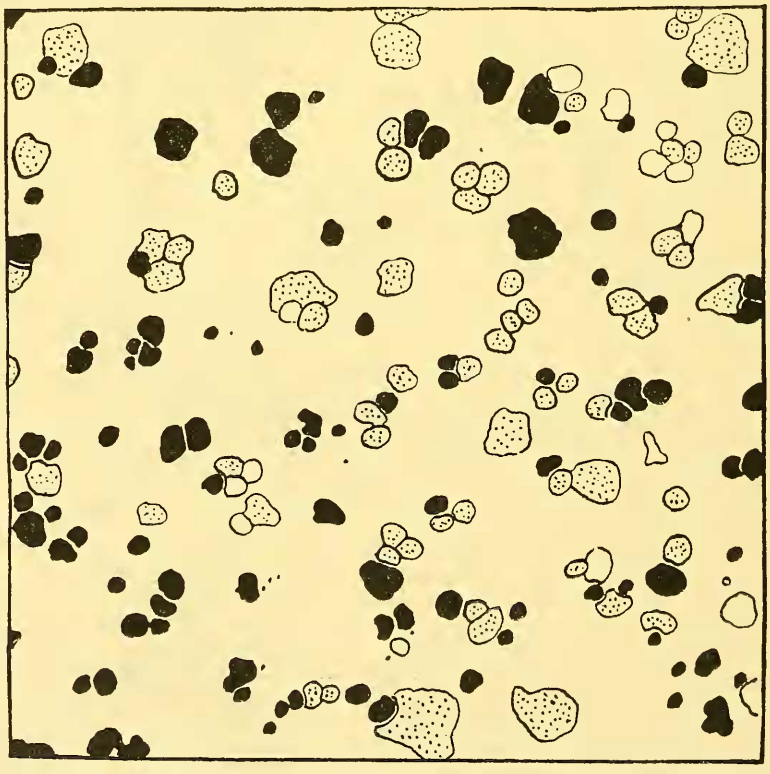


Text-figs. 2, 3.—Root systems of two plants of *Atriplex vesicarium* in plan to show the extensive development of surface roots bearing many fine lateral feeding roots, these being deciduous in drought. T.B.P. *ad nat. del.*

is the mean maximum humidity for the year at Koonamore. The regular occurrence of high maximum humidities, even during drought, is a feature of the climate at Koonamore (see below). The foliar absorption of water is possible because of the non-cuticularized leaves, and their high osmotic pressure. This Wood (1932*b*) has shown to be as high as 50 atmospheres. The high osmotic pressure is due in part to the concentration of sodium and potassium chlorides in the leaves, which ranges from 20 to 30% of the dry weight (Wood, 1925). The roots are evidently selectively permeable to these chlorides, for the soil in which they grow contains only 0·02% NaCl.

Atriplex vesicarium is a dioecious species. The fruiting bracteoles are much enlarged and in the typical form covered by a pair of convoluted bladdery appendages. There is a good deal of variation in this feature, even on the same plant. Probably more than one ecotype exists, and possibly hybridization with other *Atriplex* species occurs, but the matter will not be dealt with here.

The shrub steppe community formed by *Atriplex* is an open one. The plants generally occur in clumps or groups of individuals of all ages with bare soil between them. This gregarious habit is due to the collection of fruits and



Text-fig. 4.—Chart of a quadrat of 100 sq. metres (Salt Creek No. 1) showing canopy area of the saltbush, and gregarious habit of growth. The bare ground between the bushes was at this time well covered by the annual *Gnephosis cyathopappa*. Healthy bushes shown black, wilting and partially defoliated plants stippled, dead bushes in outline only. Plate vii, figure 3, is a photograph of this quadrat. 19.viii.1928.

wind blown soil around the established plants (cf. *Stipa nitida*, Osborn, Wood and Paltridge, 1931), and not to any capacity of the plant to reproduce vegetatively. Text-figure 4 represents a quadrat with 10-metre sides set out and surveyed in a large open paddock (Sept., 1928). It shows well the gregarious habit of the plants and the open soil between them. The number of plants recorded as almost defoliated is to be noted. Plate vii, figure 3, shows the general appearance of the quadrat. The guide strings are stretched and the area divided into metre squares.

Climatic Factors.

This section contains data as to the climatic factor relative to the growth of saltbush for the period covered by this investigation. A discussion of the full data will be given in a forthcoming paper on the general work of the Reserve.

Rainfall.

A table showing the rainfall month by month, the number of rainy days and of falls greater than 25 points, was included in our previous paper. It is sufficient to recall that the period covered by this work was one of exceptional drought and that an average annual rainfall at Koonamore (812 points) did not occur during the time of the investigation. From August, 1928, to November, 1929, both inclusive, only 176 points of rain fell. The five months, October, 1928, to February, 1929, were absolutely rainless and the next five months were without an effective fall, which we have defined as a fall of more than 25 points. A lighter fall is incapable of doing more than wet the surface of dry soil.

Temperature.

Temperature data were also given in the paper cited and need not be repeated here. The mean maximum temperature in the hot season ranges from 80° to 90° F. and from 60° to 70° in the winter. On an average for 8 years the number of frost days per annum is 53·5. A characteristic and important feature of the temperature is the high diurnal range, the mean annual range for which is 30·1° F. The greatest variation occurs during the summer months. This diurnal range has an important bearing on the humidity.

Humidity.

The humidity data are of special interest owing to the fact that high relative humidities are frequently recorded. This has an important bearing upon the water relations of *Atriplex vesicarium* because of its capacity for absorbing water through its leaves from nearly saturated air.

Relative Humidity.

Years.		Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.	Ann.
1927 to 1931	Mean Max	93	82	83	87	87	91	91	87	87	81	80	84	85
	Mean Min.	37	35	34	39	43	51	47	42	37	35	31	35	39

It will be seen that the mean maximum humidity exceeds 80% in every month of the year. On the other hand, the mean minimum humidity falls below 40% each month, except May–August, which is the winter season.

The length of time during which high humidities prevail each day is obviously a feature of importance. The following table is derived from thermo-hygrograph records taken during the three-year period 1928–1931. It shows the mean number of hours per day in each month in which the humidity is greater than 80% and also, as an indication of the more rigorous arid conditions, less than 40%.

Table showing Mean Number of Hours per Diem when Relative Humidity >80% and <40%.

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean number hours humidity >80%	Insuff. data.	3·9	4·5	7·2	9·6	8·7	8·1	6·2	4·7	3·8	3·8	3·1
Mean number hours humidity <40%		6·9	5·9	3·1	2·4	1·0	1·8	4·1	6·0	6·9	8·9	7·6

The time during which the maximum humidity is highest lies between midnight and 7 a.m., reaching a maximum usually about the time of sunrise. The time of lowest humidity is between 2 p.m. and 4 p.m.

This variation in the humidity is a consequence of the high diurnal range in temperature and, since the relative humidity varies with the temperature, a more accurate picture of the degree of saturation of the air is given by the saturation deficit. The Meyer ratios, P/sd, have been plotted for Australia by Prescott on a map showing the isologs (1931, fig. 9). The ratio for Koonamore is about 25–30.

It is more important for an understanding of the water relations of *Atriplex* to know the mean saturation deficits throughout the year. The highest relative humidity is recorded at the time of minimum temperature and vice versa. The mean saturation deficits for each month given below are therefore calculated from the mean maximum humidity and the mean minimum temperature and mean minimum humidity and mean maximum temperature respectively.

Saturation Deficits in Inches of Mercury.

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean maximum temperature ..	92·4	91·0	83·3	82·1	63·4	61·9	59·4	65·8	67·6	80·1	84·3	86·7
Mean minimum humidity ..	37	35	34	39	43	45	47	42	37	35	31	35
Saturation deficit	0·95	0·95	0·75	0·68	0·33	0·27	0·27	0·36	0·41	0·65	0·81	0·85
Mean minimum temperature ..	57·8	61·4	55·1	46·6	38·7	35·7	34·6	35·8	36·8	45·4	53·8	55·4
Mean maximum humidity ..	93	82	83	87	87	91	91	87	87	81	80	84
Saturation deficit	0·04	0·05	0·07	0·04	0·03	0·02	0·02	0·02	0·03	0·05	0·08	0·06

These figures for the saturation deficits bring out clearly that every month, even during a severe drought and during the hot summer months, the air becomes almost saturated with water vapour for a portion of each day. This fact, we believe, accounts for the success and ubiquity of the saltbush. On the other hand, the aridity of climate is indicated by the high values of the saturation deficits during the daytime.

These data as to the high relative humidity and the length of time for which it is maintained throw an unexpected light on the environmental conditions under which *Atriplex vesicarium* grows. They show that even during a drought it is more favourably situated for obtaining water than might be expected. Nevertheless, during a drought of the extreme severity experienced during these investigations, permanent wilting, defoliation or even death, is the fate of the majority of the plants. There remains to consider the effect of grazing by sheep during such a severely adverse period.

Methods.

The fundamental idea underlying these investigations upon the effect of grazing on saltbush may shortly be stated as follows. In any paddock the most intense influence of grazing and trampling by sheep (the "stock effect") will be felt around the watering place, since all the animals visit the dam or bore once or twice a day for water. Along lines radiating from the watering place the intensity of the stock effect becomes progressively less and less as the sheep pass away from the drinking place out into the paddock scattering as they go. It is a matter of observation, and so of common knowledge, that the average distance that sheep travel from water in the saltbush country is seldom greater than three miles, while the great majority are found congregated within two miles of the drinking places. During a wet winter, conditions may be different. The sheep may travel much further and remain for days in a distant portion of the paddock, depending on casual waters or the succulence of the herbage for moisture. But such conditions are exceptional. Owing to the size of the paddocks, seldom less than 4×5 miles, and often much larger, and the infrequency of the watering places (the sinking of wells or the excavation of dams is a costly process), there may be considerable areas virtually ungrazed or in a virgin state.

Around each watering place there can be defined four zones, as follows, differing from each other in the intensity of the stock effect:

1. The *A Zone*, immediately around the watering place, where inevitably the stock effect is very severe, in which the saltbushes are largely or entirely trampled out.
2. The *B Zone*, beyond A, in which the majority of the sheep feed, subjected to fairly heavy grazing and a good deal of trampling. The width of this zone depends upon the number of sheep carried in the paddock and also upon the physiography of the country around the watering place.
3. The *C Zone*, a lightly stocked area beyond the B Zone, which the sheep only visit occasionally and which is, therefore, not subjected to frequent grazing or trampling. The extent of this zone, as in the case of the B Zone, depends on the degree of stocking and on the physiography.
4. The *D Zone*, the area lying beyond the distances to which sheep normally travel. For the purposes of this investigation it has been considered as virtually ungrazed country. It affords a control or standard by which the stock effect may be gauged in the other zones.

Two desiderata were required to judge the effect of grazing upon saltbush within the four zones. These were (1) a classification of the bushes according to a scale of vegetative vigour, and (2) an inquiry into the variation in the numbers of bushes in each class of the defined scale.

As a measure of vegetative vigour, the degree of foliation of the bushes was utilized. The plants were grouped in the field into the following six classes:

1. "Dead" bushes: plants which had shed all their leaves. To determine whether a bush is really dead or not is a difficult matter, for apparently dead plants may throw up new foliage shoots after rain. As a matter of convenience all defoliated plants were grouped together. Some of these were undoubtedly dead, others moribund, but others were probably only in an anabiotic state. There is a colour difference between a dead twig and a living one. When dead the twig sheds its bark, the wood becomes dark on the outside and, when broken, is white and dry. The living defoliated twig is covered with a grey scurfy bark; when fractured it is yellowish and more or less sappy.

2. Very sparsely foliated bushes: plants with leaves on less than 20% of the shoots.

3. Sparsely foliated bushes: plants having less than 50%, but more than 20% of the shoots foliated. Vegetative vigour lacking. Leaves lacking the greenness of the turgid state, distinctly grey, even white in a bright light. Terminal shoots showing curling suggestive of wilting, well seen in the male inflorescences which bend over and become dry. Fallen leaves are common below the bush.

4. Well foliated bushes: plants with 50% or more of the twigs foliated, but lacking the uniform vigour of the next class, that of the fully foliated plants. In this class were also included certain plants which, though they have rather less than a 50% foliation, showed a vigorous development of new greenish shoots.

5. Fully foliated bushes: plants of whatever size bearing abundant foliage up to the apices of almost every twig. Leaves turgid and green-grey. Flowers or fruits, when present, turgid, the terminal male inflorescences standing erect on sappy stems. In this class were included occasional plants with a small degree of defoliation on some twigs, but vigorous growth and abundant fruit and flowers on all other branches.

6. Seedlings: in addition to seedlings proper, very young plants less than a decimetre high and obviously immature were included here.

Types of these six classes are illustrated on Plate viii. It should be noted that these photographs were taken on the same day, within a short distance of each other, also that during the preceding twelve months only 88 points of rain had fallen.

In the field the plants counted were grouped under these six headings and in the many readings made by the field officer considerable proficiency in classifying the bushes was obtained. Clearly the classes are purely arbitrary and grade into one another, but we seldom had any difficulty in deciding to which class a given bush belonged.

For the statistical treatment of the results these six classes have been reduced to four. There was an advantage in maintaining a more finely divided scale of classification in the field than was needed for the subsequent treatment of the figures. It helped to keep the observer on the alert. The four classes are:

1. Defoliated plants: plants classed as "dead" in the field notes. In presenting the results it is preferable to use a purely descriptive term which has no other implication.

2. Wilting plants: includes all those bushes listed as "sparsely foliated" and "very sparsely foliated". The remaining foliage of these plants had entered on a phase of permanent wilting, and the greater part of the plant is in the anabiotic state if not actually moribund.

3. Healthy plants: includes all plants listed as "well foliated" or "fully foliated". Such plants are obviously in a state of vegetative vigour and contrast sharply with the two preceding classes.

4. Seedlings: as defined above.

Having established a scale of vigour, a search was made for a method which would give sufficient samples of the plant population in the four zones to admit of statistical treatment. In the large areas investigated, quadrat methods are unsatisfactory. Finally a modification of the line transect was adopted. Any method adopted had to be one that a solitary observer[1] could use. Laying or stretching a tape over an extent of saltbush for a distance of two miles or more was out of the question. The method adopted, then, was as follows:

Starting from a watering place the observer took a compass bearing and walked into the paddock placing pegs at 100 pace intervals along the line of march. The pegs used were 6 feet high with the top 2 feet painted white. The lines were of variable length according to circumstances. Some extended as much as 2½ miles from the watering place, others less. The ungrazed country, classified as D Zone, lay beyond the ends of the transects, 4 to 5 miles from the water.

The unit of length adopted was the observer's full stride, which we term the pace. This is a long one, 1·5 yards (54 inches). The full stride was deliberately adopted because it was found easier to maintain this at a constant length when continually stopping and starting than any arbitrary pace.

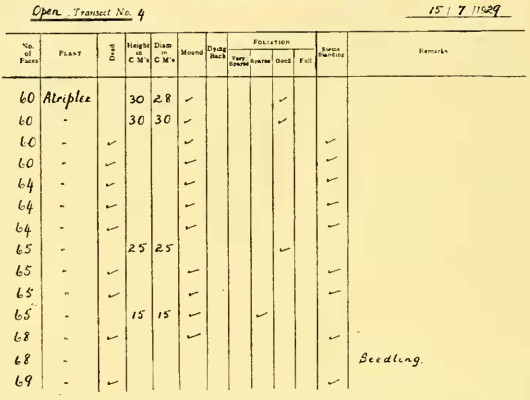
Having established his line of pegs the observer would return to the starting point and "pace" along the line, always keeping two or more pegs in line before him. On specially prepared record sheets he entered every bush which was *actually crossed* by his line of march, the unit being the pace. Notes were also made as to the vigour and size of each bush recorded. The record sheets were uniform foolscap size; portion of one, transcribed from an actual page, is shown in Text-figure 5.

It will be seen that the transect is really a strip transect of a width determined by the observer, who had to judge whether or not a particular bush was actually cut by his line of march. Obviously there might be a certain amount of selection but, since all records were made by the same individual, the error, if such there be, is likely to be uniform. The results are remarkably consistent, e.g., the D Zone in St. Patrick's was surveyed twice, in August, 1929, and November, 1930, when the mean number of plants per 100 paces was 181 ± 19 and 181 ± 21 respectively.

From each watering place three or four such line transects were laid down radiating into the paddock. It was by no means easy to get clear runs of salt-

[1] This is a fitting place to pay tribute to the energy and enthusiasm of the field officer, T. B. Paltridge, who lived three years at the Koonamore Vegetation Reserve. Paltridge set out and surveyed all the transect lines described in this paper as well as several other systems not mentioned here. We have jointly and individually visited most of the transects with him more than once and have discussed the work with him in the field at all stages of its progress. To Paltridge, however, fell the task of recording the data under difficult conditions and sometimes with no small physical discomfort.—T.G.B.O. and J.G.W.

bush about 2 miles long without some physiographic variations, i.e., flooded areas, watercourses or rocky outcrops, and at the same time avoid feeding grounds from other waters. The five transect systems described are free from objections of this type. In the case of the Melton transects to be described later, a variation



St Patrick's Paddock — Page 4

Open — Transect No. 4 — 15/7/1929

No. of Paces	Plant	Dead	Height in C.M's	Diam in C.M's	Mound	Dying Back	Foliation: Very Sparse	Foliation: Sparse	Foliation: Good	Foliation: Full	Bunch Shedding	Remarks
60	Atriplex		30	28	✓				✓			
60	-		30	30	✓				✓			
60	-	✓			✓						✓	
60	-	✓			✓						✓	
64	-	✓			✓						✓	
64	-	✓			✓						✓	
64	-	✓			✓						✓	
65	-		25	25					✓			
65	-	✓			✓						✓	
65	-	✓			✓						✓	
65	-		15	15	✓			✓				
68	-	✓			✓						✓	
68	-											Seedling
69	-	✓									✓	

Text-fig. 5.—Portion of an actual record sheet.

of the line transect was used. The original line from the watering place was laid down and counts made along it as usual, but, in addition, at every 200-pace interval a cross transect at right angles to the main transect was run for 300 paces. This method is considered to be superior to the system of radiating lines, as more samples within the different zones can be obtained.

For comparison of the different classes of plants in the different zones, the number of bushes per 100 paces was treated as a unit and sheets drawn up showing the total number of plants, the number of defoliated, of wilted, of healthy plants and of seedlings per 100 paces.

There were now available for each zone a number of 100-pace unit samples of the population and from these figures the following statistics were calculated for each class: the mean per 100 paces, the standard deviation of the mean, and the standard error of the mean. Comparisons of the mean number of plants of any class in different zones could then be made by use of the difference of means and their standard error of difference, a difference being regarded as significant if the difference of the means exceeded twice the standard error of difference. A standard error of grouping was also calculated and the grouping is sufficiently fine in all cases dealt with except in that of the seedlings, which, unless otherwise mentioned, are not considered in the following discussion of the results. Sufficient samples were obtained to take into account any variations due to physiographic influences.

Results.

In the following tables the $\pm$ sign is given before the standard deviation and standard error of the means, and also the standard error of difference, in the first line only. It is to be understood as referring to all figures in the respective columns. In the columns giving the difference of the means a significant difference